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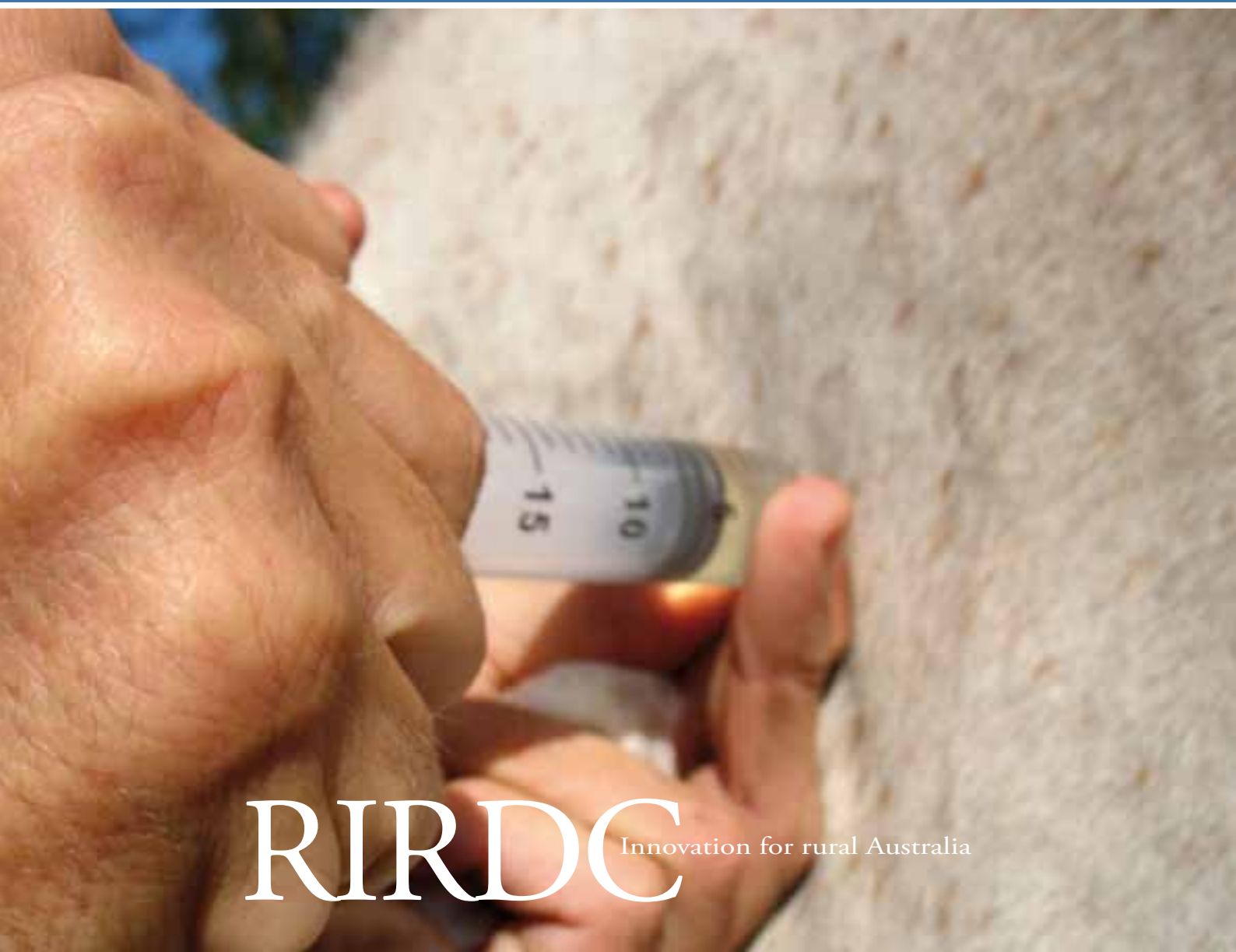
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The Pharmacokinetics of Equine Medications

RIRDC Publication No. 11/117



RIRDC Innovation for rural Australia



Australian Government

**Rural Industries Research and
Development Corporation**

The Pharmacokinetics of Equine Medications

by

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Foreword

The Australian horse industry is significant by world standards, with thousands of horses competing each year in flat racing, harness racing and equestrian events. Illness and training injuries are common however treatment is affected by uncertainty about excretion and detection times for many therapeutic drugs. Owners and trainers in such a valuable industry require access to the highest quality information on the management of medications.

By investing in this project the Rural Industries Research and Development Corporation has supported a consortium of Australia's leading forensic chemists, veterinarians and equine scientists, generating significant capacity to address this problem on a scale never before undertaken in Australia.

The project has involved widespread industry consultation and has been particularly rigorous in selecting the compounds for study, according to the most urgent needs and demands of veterinarians. The investigative team has sought to raise the standard of research in this field by employing state-of-the-art scientific methodology, an appropriate number of horses to yield conclusive results, and advanced data modelling techniques to estimate the likely variation in the horse population as a whole.

The figures, tables and pharmacokinetic data in this report should be immediately useful to veterinarians when designing appropriate treatment protocols for sick and injured horses. The data concerning drug excretion times is currently being reviewed by the Australian racing authorities and will inform the publication of reliable detection times for these therapeutic substances. Finally, this information will be shared with the relevant authorities in Asia, Europe and the USA, reducing the need for unnecessary duplication of effort and contributing to the process of harmonisation of drug detection protocols worldwide.

This project was funded by voluntary industry contributions and RIRDC government funds allocated to the Horse Program. Additional funding was supplied by the project participants and by a number of direct industry sponsors as identified in the acknowledgements section.

This report is an addition to RIRDC's diverse range of over 2000 research publications and it forms part of our Horse R&D program, which aims to improve the safety of industry participants and the welfare of horses, and enhance the environmental sustainability of the industry.

Most of RIRDC's publications are available for viewing, free downloading or purchasing online at www.rirdc.gov.au. Purchases can also be made by phoning 1300 634 313.

Craig Burns

Managing Director

Rural Industries Research and Development Corporation

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Abbreviations

See Glossary for list of abbreviations.

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Executive Summary

What the report is about

This report provides information about the pharmacokinetics of 12 important equine medications. For each medication, the report illustrates that time taken for the drug to reach its maximum concentration (and hence maximum effectiveness) in the blood, its rate of metabolism and removal from the bloodstream, and the time taken for the drug and its metabolites to be eliminated from the body via the urine.

The report also provides new insights into how metabolite data can be modelled to provide useful information about the behaviour of the parent drug, and how the variation between individual horses can be measured and used to estimate the level of risk of any horse in the population of breaching a detection limit at any given time point after drug administration.

This information should assist veterinarians to develop more effective treatment protocols for horses, and will assist the racing industry regulators in setting appropriate detection times and reporting levels for therapeutic substances in animals that compete.

Background

Thousands of horses compete each year in flat racing, harness racing and equestrian events. Illness and training injuries are common, but appropriate treatment can be hampered due to uncertainty about excretion and detection times for many therapeutic drugs.

This uncertainty stems from the fact that current information about excretion times is based on very limited data, sometimes obtained from only one or two horses. Furthermore, although we know that different horses may metabolise and excrete the same drug at different rates, there is no information about the size of this variation, and hence the risk of basing a withholding time for a particular horse on an ‘average’ detection time observed in an experimental study.

To address this problem, the four Australian racing laboratories have joined forces with four universities under the banner of Equine Therapeutics Research Australia (ETRA). Over a four-year period the team has conducted 18 drug administration trials using 12 horses in most trials, developed new analytical methods, and processed more than 20,000 blood and urine samples. This report describes the results obtained from 12 of these trials.

Aims/objectives

The aims and objectives of the project were to:

- identify the most important therapeutic substances used in horses;
- administer these compounds to groups of not fewer than 10 horses, collect plasma and urine samples and analyse these for the presence of the parent drugs and their metabolites;
- model the analytical data, presenting a clear summary of the pharmacokinetics and rate of excretion of each compound for the benefit of veterinarians and racing authorities;
- explore a new approach to the reporting of detection times based on a probability model which takes into account variation among horses.

Methods

The test substances were selected after widespread consultation with the industry stakeholders and veterinarians. The drugs were administered to groups of 10 to 12 horses housed either at Charles Sturt University, NSW, or The University of Queensland, Gatton, Qld. Blood and urine samples were collected at frequent intervals over an appropriate period of time, ranging from 5 days to 100 days depending on the predicted excretion time of the drug. The samples were transported to one of the four Australian racing laboratories where they were subjected to rigorous chemical analysis using fully validated protocols and advanced instrumentation. The analytical data was then modelled to determine the pharmacokinetic parameters for each drug. A Bayesian probability model was used to investigate one drug in depth (acepromazine), to account for variation between horses and develop a risk-based approach to judging an appropriate withholding time.

Results/key findings

A summary of the results is shown in Table 1.

Table 1 Peak plasma concentrations and excretion times for 12 equine medications

Compound	Route ^a	Dose	n ^b	T _{max} ^c	E ^d
Phenylbutazone	oral	2 g twice daily for 1 day then 1 g twice daily for 4.5 d (1.5 to 2 mg/kg twice daily)	12	4.3 h	>9 days
Flunixin	IV	1.1 mg/kg	12	5 min	>10 days
Ketoprofen	IV	1 g/horse (1.86 to 2.29 mg/kg)	12	5 min	3 days
Hydrocortisone	IV	1 g /horse (1.55 to 2.02 mg/kg)	10	18 min	36 h
Prednisolone	oral	1 g/day for 5 days (~2 mg/kg)	12	40 min	5 days
Methylprednisolone	IM	200 mg/horse (0.39 to 0.47 mg/kg)	12	36 min	10 weeks
Acepromazine	IV	30 mg/horse (0.045 to 0.063 mg/kg)	12	5 min	>6 days
Detomidine	IV	0.04 mg/kg	12	3 min	3 days
Buscopan	IV	30 mL/horse (120 mg HBB, 0.21 to 0.27 mg/kg and 15 g dipyrone, 26 to 33 mg/kg)	12	5 min	13 days
Mepivacaine	SC	400 mg/horse (0.68 to 0.99 mg/kg)	12	1 h	>4 days
Lignocaine	SC	0.8 mg/kg	12	30 min	5 days
Prilocaine	SC	400 mg/horse (0.84 to 0.60 mg/kg)	12	35 min	3 days

^aIV - intravenous injection, IM - intramuscular injection, SC - subcutaneous injection; ^bn - number of horses used; ^cT_{max} - time after administration at which plasma concentrations of the parent drug peak; ^dE - time after administration at which no drug or metabolite was detected in blood or urine in any horse (NB - does **not** represent official detection time).

Implications for relevant stakeholders

The results of this report will allow veterinarians to develop improved therapeutic protocols which will benefit all horses, including the many thousands of horses across Australia treated each year for illnesses and injuries, which never compete.

In addition, these data will assist the racing authorities in setting more appropriate, accurate and reliable detection and reporting times for therapeutic substances. This information will subsequently benefit the veterinarians, owners and trainers associated with more than 650,000 starters in Thoroughbred racing, harness races and other equestrian pursuits which compete in Australia each year.

Finally, as the information will be available to racing jurisdictions world-wide, this will assist Asian, European and American authorities in avoiding duplication of effort and moving towards a harmonised approach to drug detection.

Recommendations for further action

- Completion by the ETRA group of the analysis of those compounds currently under investigation, and the complete study published in a comprehensive handbook;
- Cooperation between the ETRA group and racing authorities to develop appropriate detection times and reporting levels for the therapeutic substances, with the resultant guidelines made accessible for the equine industry;
- Exploration of the Bayesian probability approach to guide clinicians towards appropriate withholding times for other therapeutic drugs, particularly those with an excretion time of longer than 48 hours;
- Presentation of the results of this study published as a series of scientific papers and presented at international conferences;
- Strengthened engagement by the ETRA group with its counterparts in Asia, Europe and North America, with a view to making more information available for Australian users.

Introduction

The Australian horse industry comprises thousands of horses competing each year in flat racing, harness racing and equestrian events. Illness and training injuries are common, but there is uncertainty about excretion and detection times for many therapeutic drugs.

In some cases a horse may be treated for a legitimate purpose and due to an error of judgement, may test positive on race day for a prohibited substance. This may lead to sanctions for the owner and/or trainer, damage to the reputation of the veterinarian, and compromises the integrity of the sport. Conversely, a horse in need of medication may be denied therapy for fear of breaching drug detection limits, to the detriment of the horse's welfare. Finally, regardless of whether a horse is a competition animal or not, *"there is a critical need for reliable information about the pharmacokinetics (PK) of veterinary medications to assist the clinician in using these drugs to their best effect"* (Barry Smyth, former President, Equine Veterinarians Australia).

The source of uncertainty about drug detection times lies in the way that drug excretion rates are currently estimated. Independent racing forensic laboratories in each state typically maintain two or three horses, and employ a veterinarian to conduct drug administration trials on an *ad hoc* basis. Urine samples are collected and analysed by the laboratory to estimate the disappearance rate of the drug from the horse's system. Based on these data, a suggested detection period is published by Equine Veterinarians Australia in the 'White Book', which is available to its members.

This approach is inefficient, as there is a significant cost to each laboratory for maintaining the horses, using the services of a veterinarian, and engaging an animal ethics committee to approve each study. Moreover, only a rough estimate of an average drug excretion rate is obtained, as each study is limited to three (and sometimes even one or two) horses. Because the likely variation between different horses cannot be estimated, clinical decisions are hampered and detection times cannot be generalised to the entire horse population with much confidence. Finally, much information in the White Book is now out of date.

With financial support from industry stakeholders, a consortium of the four Australian racing laboratories and four universities was formed to address this problem under the banner of Equine Therapeutics Research Australia (ETRA). The universities have jointly provided access to 24 horses, PK modelling and statistical expertise; while the laboratories have employed their analytical skills to process more than 20,000 blood and urine samples, as well as developing new forensic tests by investigating the pharmacodynamics (PD) and metabolism of selected compounds.

Predicted benefits

Using a large number of horses to test each drug has increased the accuracy of the estimated PK parameters and average excretion rates, and has generated important information about the variation between horses, which has been difficult to gauge from earlier work using fewer animals. Based on this variation, we have explored a different approach to estimating detection and withholding times by the application of Bayesian statistics. Using this method we have demonstrated that it is possible to report the probability of an individual horse returning a positive swab at any given time-point after treatment. Compared to the current system, this is an innovative and more realistic approach to the problem of drug detection times, which is essentially one of risk management.

Additional benefits from this project will accrue over time as further compounds are analysed, the data are reviewed by the racing authorities, and new recommendations about detection times are published. The ETRA group is also consulting with its counterparts in Asia, Europe and America, to share data, maximise the information available to Australian stakeholders, avoid duplication of effort and contribute to the international harmonisation of drug testing protocols.

Objectives

The objectives of this project were as follows.

- To identify the most important therapeutic substances in each of several categories from the standpoint of frequency of use, frequency of positive drug tests and lack of reliable information concerning their pharmacokinetics and excretion times.
- To administer the selected compounds to groups of not fewer than 10 horses, then collect plasma and urine samples with appropriate frequency and over an extended period of time, for forensic analysis.
- To analyse the equine samples for the presence of the parent drugs and their metabolites, and in certain cases develop new analytical methods
- To process and model the analytical data, presenting a clear summary of the pharmacokinetics of each compound
- To provide drug and metabolite excretion data to the relevant horseracing authorities so that appropriate reporting levels and detection times can be calculated and made available to the industry
- To explore a new approach to the reporting of detection times based on a Bayesian probability model which takes into account variation among horses.

Methodology

Selection of test substances

The test substances were selected after a four-stage process involving widespread industry consultation. Firstly, a list of candidate substances was prepared by the project management committee which included members from each of the four Australian racing laboratories, a representative from Equine Veterinarians Australia and a representative from each of the three participating universities.

Candidate substances were considered against several criteria including the frequency of positive drug tests and the quality of information about their pharmacokinetics or excretion times already available in the international scientific literature or elsewhere.

The second stage involved consultation with a reference group comprising representatives from the following industry bodies:

- The Australian Horse Industry Council
- Australian Racing Board
- Racing and Wagering Western Australia
- Racing Queensland
- Racing New South Wales
- Racing Victoria,
- Harness Racing Australia
- Equestrian Australia
- Veterinary Manufacturers and Distributors Association.

Stage three involved surveying equine practitioners at the Bain Fallon Conference (Cairns, Qld 2008). Forty three equine veterinarians completed a questionnaire naming the therapeutic substances they used most frequently, specifying the usual dosage and route of administration, and scoring on a scale of 1 to 5 their need for more accurate information about excretion times for these drugs.

Stage four was the final part of the selection process where the management committee reviewed the information provided by the survey and the various stakeholders. The final list was prepared (Tables 1 and 2) and the administration trials and analyses were allocated to the various participating universities and laboratories.



Figure 1 Ten of the horses used to determine excretion times for equine medications

At the time of writing, 18 drug administration trials have been performed. Table 2 lists those compounds which have been administered, analysed and included in this report. Table 3 lists the compounds which have been administered, but which are still undergoing chemical analysis by the racing laboratories.

Table 2 Twelve therapeutic substances administered to groups of 10 or 12 horses (n) to determine their pharmacokinetics in blood and excretion times in urine

Class	Compound	Route*	Dose	n
Non-steroidal anti-inflammatory drugs	Phenylbutazone	oral	2 g twice daily for 1 day then 1 g twice daily for 4.5 d (1.5 to 2 mg/kg twice daily)	12
	Flunixin	IV	1.1 mg/kg	12
	Ketoprofen	IV	1g/horse (1.86 to 2.29 mg/kg)	12
Corticosteroids	Hydrocortisone	IV	1 g /horse (1.55 to 2.02 mg/kg)	10
	Prednisolone	oral	1 g/day for 5 days (~2 mg/kg)	12
	Methylprednisolone	IM	200 mg/horse (0.39 to 0.47 mg/kg)	12
Sedatives	Acepromazine	IV	30 mg/horse (0.045 to 0.063 mg/kg)	12
	Detomidine	IV	0.04 mg/kg	12
Antispasmodics	Buscopan	IV	30 mL/horse (120 mg HBB, 0.21 to 0.27 mg/kg and 15 g dipyron, 26 to 33 mg/kg)	12
Local anaesthetics	Mepivacaine	SC	400 mg/horse (0.68 to 0.99 mg/kg)	12
	Lignocaine	SC	0.8 mg/kg	12
	Prilocaine	SC	400 mg/horse (0.84 to 0.60 mg/kg)	12

*IV –intravenous injection, IM – intramuscular injection, SC – subcutaneous injection

Table 3 Four therapeutic substances administered alone or in combination to groups of 12 horses (n) to determine their pharmacokinetics in blood and excretion times in urine – currently under investigation

Class	Compound	Route*	Dose	n
Corticosteroids	Dexamethasone	IV	0.06 mg/kg	12
Analgesics	Butorphanol	IV	20 mg/horse	12
Tranquilizers	Xylazine	IV	400 mg/horse	12
	Xylazine + butorphanol	IV	250 mg xylazine/horse 10 mg butorphanol/horse	12
Antibiotics	Procaine penicillin (1d)	IM	12 mg/kg single administration	12
	Procaine penicillin (5d)	IM	12 mg/kg daily for 5 days	12

*IV –intravenous injection, IM – intramuscular injection

Animals and husbandry

A group of 12 Standardbred and Thoroughbred horses, purchased at local saleyards, was housed at the Equine Centre, Charles Sturt University, Wagga Wagga NSW (CSU). A second group of 12 horses, all Standardbred, was purchased from a dealer after retirement from racing and was housed at the Equine Unit, The University of Queensland, Gatton Campus, Qld (UQ). This duplication of herds allowed the project to progress in a timely manner and reduced the risks associated with an outbreak of infectious disease. Both herds were subjected to the same treatment and husbandry procedures unless stated otherwise.

The horses were all geldings of racing age and were kept on pasture for most of the time. During trial periods when the horses were stabled they were exercised for one hour each day on a mechanical horse walker. Their diet comprised either mixed pasture *ad libitum*, or lucerne or other good-quality hay while they were confined, fed at approximately 2% of bodyweight per day to maintain a moderate body condition score of 3 on a scale of 0 to 5.

The project lasted for four years and individual horses in each herd were replaced from time to time. Thus, the mean age and bodyweight of each treatment group varied, but was approximately 8 years and 520 kg.

Throughout the project the horses were monitored by registered veterinarians and received regular foot and dental care as well as treatment for internal parasites. Any medications administered for minor injuries or illnesses were recorded.

General treatment and sampling protocol

All experimental procedures were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC, 2004) and were approved by the Animal Care and Ethics Committees of the University of Queensland and Charles Sturt University.

Nine drug administration trials were performed at each University to examine the pharmacokinetics and urinary excretion times of 16 therapeutic substances given alone or in combination. Each administration trial involved 12 horses apart from 1 study which used 10 horses. This number was designed to yield more accurate data than those obtained previously in smaller scale studies.

For each trial the horses were brought into the stables for several days before drug administration, allowing them to adjust to their new dietary, housing and exercise regimen. On the day of treatment, catheters (14 G, Angiocath, Becton Dickinson, Franklin Lakes, NJ, USA) were inserted into the

jugular vein of each horse after the administration of a local anaesthetic (0.5 to 1.0 mL, Ilium Lignocaine 20, Troy Laboratories Australia Pty Ltd, Smithfield, NSW, Australia). The horses were also fitted with specially-designed harnesses (Figure 1) which allowed the collection of all urine into a 2 L plastic container that was emptied as necessary.



Figure 2 Horse wearing a harness designed for the collection of urine

Pre-treatment blood and urine samples were collected via the venous catheter and urine collection bottle before each horse was given the therapeutic substance at a prescribed dose rate and via the route of administration specified in Table 1. Further blood and urine samples were collected at intervals which were slightly different for each drug depending on the expected excretion profile. The trials typically involved the collection of blood samples at 5 to 10 min intervals for the first hour, 20 to 30 min intervals for the next 2 h, hourly intervals for up to 8 h, then either daily or twice daily for the remainder of the trial. Several substances were expected to be fully excreted within 48 h, whereas the substance with the slowest excretion rate (methylprednisolone) involved sampling for up to 100 days.

Blood samples were collected into 10 mL lithium heparin Vacutainers (Terumo, Somerset, NJ, USA), and centrifuged at 4 °C (10 min; 2,800 x g). Plasma was harvested and stored at -20 °C until it was transported on dry-ice, to one of four Australian racing laboratories for analysis.

Urine samples were collected less frequently than blood, typically at intervals of 2 h for the first 12 h, then daily until the end of the trial. Urine was also stored frozen at -20 °C until transported for analysis.

Chemical analyses

All blood and urine samples were analysed by official Australian horseracing forensic laboratories. Standard analytical methods and instrumentation were used (usually LC-MS or GC-MS), following an appropriate sample extraction procedure. All methods were fully validated involving the use of appropriate calibration standards and quality control samples. The details of each procedure are included in subsequent chapters of this report.

Pharmacokinetic modelling

The pharmacokinetic data were analysed using WinSAAM software, (<http://www.winsaam.com>, University of Pennsylvania, PA, USA) and STATA Statistical Software 11.1 (Stata, 2007) according to the methods described by Stefanovski et al. (2003).

The $\log_{10}$ of the plasma drug concentrations were plotted against time and the resultant curves were resolved into exponential (linear) components. The areas under the plasma-concentration versus time plots (AUC) were calculated on the basis of the trapezoidal rule and extrapolated to infinity using the slopes of the elimination phase.

The volume of distribution (Vd) was calculated according to the equation $Vd = \text{dose}/\text{initial concentration of drug in plasma}$. The volume of distribution at steady state (Vssc) was calculated according to the equation $Vssc = \text{dose} * \text{MRT}/\text{AUC}$.

Based on the pharmacokinetic data the times for plasma drug concentrations to fall to a designated level were determined using the equation $\text{Time (T)} = (1/\beta) * \log B / \text{concentration}$, where concentration is the designated level in $\mu\text{g/L}$. Designated plasma concentrations chosen were 1.0, 0.1 and 0.01 $\mu\text{g/L}$. The designated levels chosen are arbitrary and in no way reflect any reporting levels that may be used by forensic laboratories.

All data are presented as the mean $\pm$ standard error (se) or standard deviation (SD) as indicated and a *P*-value of < 0.05 was used to determine significant associations.

Pharmacokinetic modelling - mepivacaine

In contrast to the other therapeutic substances described in this report, an initial examination showed that the pharmacokinetics of mepivacaine are non-linear, and so a different modelling approach was adopted. This approach is described in detail in Chapter 9.

1 Phenylbutazone

Background

Phenylbutazone is an acidic, lipophilic non-steroidal, anti-inflammatory drug (NSAID) that is widely used in equine veterinary medicine to treat conditions associated with pain and inflammation, such as musculoskeletal disorders (Hubbell et al., 2010). The drug has analgesic, anti-pyretic and anti-inflammatory properties and works by inhibiting the cyclo-oxygenase (COX) enzyme pathway, thereby preventing the release of inflammatory mediators including prostaglandins, prostacyclin and thromboxane (Beretta et al., 2005).

The drug is available in both oral and parenteral (intravenous (IV)) preparations and is well absorbed following oral dosing (Jeffcott and Colles, 1977). Oral preparations are available as granules (Butalone, Apex Laboratories; Deltazone Equine, Ceva Delvet; Equibutazone, Virbac Australia and Beautamav, Mavlab) and paste (Bute, Ranvet; Butin Anti-inflammatory Oral Paste, International Animal Health and P-Butazone, Vetsearch International). Common dose rates for oral preparations in horses are 2 g once daily on the first day then 1g once a day for four consecutive days (eMIMS IVS Australia, 2011). Dose rates are generally lower in ponies (e.g. 1 g daily for four days). However, dose rates vary depending on the product and formulation, so close adherence to the recommended dosage schedule provided with each individual product is required. Products available for parenteral administration are given by slow intravenous injection (2.2 to 4.4 mg/kg) and include Butasyl (Novartis Animal Health), Salbute Injection (Nature Vet) and Ilium Nabudone P (Troy Laboratories).

Phenylbutazone is an enolic acid and is highly protein bound (> 99%), but penetrates well into inflamed tissue due to local increases in blood flow (Lees et al., 2004; Gandal et al., 1969).

Phenylbutazone is contra-indicated in horses with gastric or oral ulceration, hypoproteinaemia, blood dyscrasias, or pre-existing renal, hepatic or cardiac disorders (MacAllister et al., 1993; Moses and Bertone, 2002; Radi and Khan, 2006).

Feeding adversely affects phenylbutazone absorption following oral administration and the consumption of hay can significantly delay the time of peak plasma concentration and reduce urine concentrations of phenylbutazone and its metabolites during the initial 24 h after oral dosing (Lees et al., 1987; Tobin et al. 1986). It is eliminated mainly by hepatic metabolism and excreted via the kidneys either unchanged, or as its major metabolites oxyphenbutazone, γ -hydroxyphenylbutazone and γ -hydroxyoxyphenbutazone (Gerring et al., 1981; Moses and Bertone, 2002). More rapid excretion can be observed in alkaline as opposed to acidic urine (Moss and Haywood, 1973).

Studies on the excretion time of phenylbutazone following IV administration in racehorses have been performed previously, and have found that plasma clearance is slightly decreased in horses that have been exercised immediately after treatment (Authie et al., 2010). However, to the authors' knowledge, published studies on the PK of phenylbutazone after oral dosing have not been performed since the 1980's (Lees et al., 1987). In the present study, phenylbutazone paste was administered orally to 12 horses over a period of 5 days and the plasma and urine concentrations of the drug and its metabolites were analysed both during and after treatment.

Phenylbutazone administration

Phenylbutazone (Bute Paste, Ranvet) was administered orally (2 g twice daily (bid) on the first day then 1 g bid for 4 consecutive days, followed by 1 g once on day 6) to 12 mature geldings (4 Thoroughbred, 5 Standardbred; mean body weight 564 ± 10.9 kg) housed at Charles Sturt University, NSW.

Blood (20 mL) and urine (40 mL) samples were collected before the first dose of phenylbutazone was administered and then each morning prior to dosing for the 6 day treatment period. Following the last oral dose of phenylbutazone blood and urine samples were also collected at frequent intervals throughout day 1, twice on the second day after treatment, and then daily for 8 days (Table 1.1).

Immediately after collection, blood samples were centrifuged ($2,800 \times g$, 20 min). The plasma was divided into 2 portions and stored in plastic vials at -20°C . Urine samples were also divided into two portions and stored at -20°C . All samples were transported frozen to Racing Analytical Services, Vic., where they were analysed for the presence of phenylbutazone and its metabolites.

Extraction and analysis procedures

Calibration curves for phenylbutazone and its metabolite oxyphenbutazone in urine and blood were constructed over the range 0 to 2000 ng/mL using phenylbutazone- d_{10} as the internal standard. Phenylbutazone and oxyphenbutazone were extracted from equine urine using a simple liquid/liquid extraction. Urine was spiked with phenylbutazone- d_{10} as an internal standard, sodium acetate buffer was added to adjust the pH to 3, and the urine was then extracted with trisolvent (hexane/dichloromethane/ethyl acetate). The organic layer was removed after extraction, evaporated to dryness and reconstituted in methanol/Methelute[®] for on-column derivatisation.

A simple liquid/liquid extraction procedure was also employed for plasma. The plasma samples were adjusted to acid pH by the addition of dilute HCL and extracted with diethyl ether. The organic layer was removed, evaporated and the residue was reconstituted in methanol/ Methelute[®] for on-column derivatisation.

Both urine and plasma extracts were analysed using GCMS. An SGE BPX5 column was used for chromatography with a temperature gradient from 75°C to 300°C . A selected ion monitoring method was used to acquire data; three ions were monitored m/z 322.1 (methylated phenylbutazone), m/z 332.1 (methylated D10-phenylbutazone) and m/z 352.1 (methylated oxyphenbutazone).

Pharmacokinetic modelling

The pharmacokinetics of phenylbutazone were modelled at Charles Sturt University, NSW and The University of Pennsylvania, PA, using WinSAAM software (<http://www.winsaam.com>, University of Pennsylvania) and STATA Statistical Software 11.1 (Stata, 2007) as described in the general methods section. PK data are presented as the mean $\pm$ SD. All other data are presented as mean $\pm$ se.

Results and discussion

Plasma phenylbutazone

Plasma concentrations of phenylbutazone and its metabolite, oxyphenbutazone (OPB), are shown in Table 1.1 and Figure 1.1. During the 5 day treatment course, phenylbutazone concentration peaked on the second day of the treatment period at 6078 ng/mL, and then remained stable for the remaining 3 days (Fig. 1.1).

Table 1.1 Plasma and urine phenylbutazone concentrations (mean $\pm$ se) during and following 5 days of oral dosing (2 g bid on day 1 then 1 g bid for 4.5 d) in 12 horses

Time	Plasma PBZ (ng/mL)	Plasma OPB (ng/mL)	Urine PBZ (ng/mL)	Urine OPB (ng/mL)
-5 days	0	0	0	0
-4 days	6078 $\pm$ 901	1061 $\pm$ 131	7273 $\pm$ 597	20066 $\pm$ 2526
-3 days	3784 $\pm$ 322	851 $\pm$ 131	5439 $\pm$ 297	18086 $\pm$ 1897
-2 days	3042 $\pm$ 195	751 $\pm$ 65.6	5065 $\pm$ 410	22900 $\pm$ 5438
-1 days	3261 $\pm$ 197	652 $\pm$ 50.9	6913 $\pm$ 563	27073 $\pm$ 2602
0 h	3127 $\pm$ 176	570 $\pm$ 25.5	7348 $\pm$ 506	27555 $\pm$ 4131
0.25 h	3271 $\pm$ 293	441 $\pm$ 31.2	-	-
0.5 h	5687 $\pm$ 1119	449 $\pm$ 40.8	-	-
0.75 h	5423 $\pm$ 749	1057 $\pm$ 54.9	-	-
1 h	5701 $\pm$ 794	1113 $\pm$ 66.8	-	-
1.5 h	8587 $\pm$ 1007	533 $\pm$ 46.5	-	-
2 h	10282 $\pm$ 1138	598 $\pm$ 48.8	10462 $\pm$ 833	42077 $\pm$ 6417
2.5 h	11279 $\pm$ 1362	712 $\pm$ 62.1	-	-
3 h	9828 $\pm$ 1156	975 $\pm$ 74.0	-	-
4 h	11558 $\pm$ 1185	1050 $\pm$ 71.4	10217 $\pm$ 1384	21154 $\pm$ 1819
6 h	10296 $\pm$ 592	1099 $\pm$ 92.5	9660 $\pm$ 781	28257 $\pm$ 3902
8 h	6321 $\pm$ 338	1193 $\pm$ 78.3	9208 $\pm$ 715	31250 $\pm$ 4238
12 h	3354 $\pm$ 232	1029 $\pm$ 92.5	6399 $\pm$ 333	22525 $\pm$ 2362
18 h	-	-	4407 $\pm$ 315	20766 $\pm$ 1349
24 h	799 $\pm$ 69.4	209 $\pm$ 24.5	2462 $\pm$ 240	13015 $\pm$ 1908
28 h	-	-	1341 $\pm$ 176	8996 $\pm$ 1625
32 h	448 $\pm$ 53.2	127 $\pm$ 13.1	778 $\pm$ 107	5437 $\pm$ 1364
38 h	-	-	771 $\pm$ 88.9	4000 $\pm$ 388
42 h	-	-	332 $\pm$ 43.1	2091 $\pm$ 267
48 h	96.1 $\pm$ 11.8	40.4 $\pm$ 3.03	239 $\pm$ 33.8	1561 $\pm$ 284
72 h	31.8 $\pm$ 2.31	26.4 $\pm$ 0.60	18.3 $\pm$ 3.24	101 $\pm$ 25.2
96 h	12.6 $\pm$ 1.21	10.4 $\pm$ 0.38	16.9 $\pm$ 2.30	39.7 $\pm$ 3.32
120 h	7.08 $\pm$ 0.71	3.69 $\pm$ 1.32	25.4 $\pm$ 2.41	32.1 $\pm$ 1.20
144 h	5.65 $\pm$ 0.36	0	2.07 $\pm$ 0.67	6.99 $\pm$ 1.30
168 h	3.38 $\pm$ 0.19	0	1.23 $\pm$ 0.56	5.22 $\pm$ 1.73
192 h	2.42 $\pm$ 0.31	0	2.80 $\pm$ 0.99	14.7 $\pm$ 2.07
216 h	0	0	0.99 $\pm$ 0.47	10.0 $\pm$ 0.89

Following the treatment course, phenylbutazone levels appeared to show a double peak 2.5 h and 4 h after the last dose. This twin peak phenomenon has been observed previously and is thought to be due to rapid absorption in the acidic environment of the stomach and small intestine, influenced by gastric emptying, with the remaining phenylbutazone being absorbed into some component of the feed (Maitho et al., 1986). Often the initial peak is disregarded for PK analysis and this was so for the present preliminary analysis.

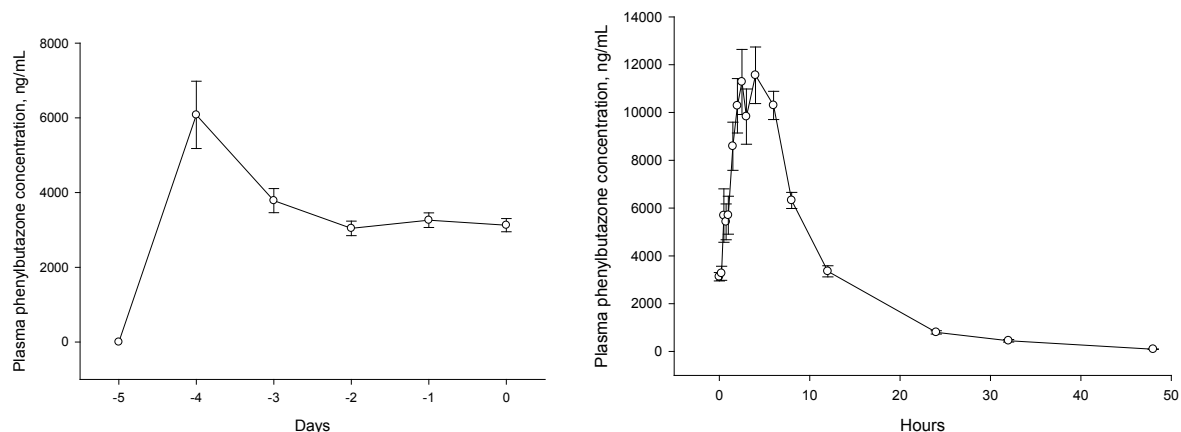


Figure 1.1 Plasma concentrations of phenylbutazone (mean $\pm$ se) during and after a 5 day course of phenylbutazone administered orally to 12 horses commencing on day -5

After the second peak, plasma phenylbutazone concentrations subsided rapidly and were minimal 48 h after treatment (Fig 1.1). However, plasma phenylbutazone concentrations were still detectable in plasma in 11 of the 12 horses for up to 8 days (192 h). After 9 days (216 h) plasma phenylbutazone was not detectable in any horse.

The PK analysis of phenylbutazone showed that the data fitted a 3-compartment model, with distinct absorption, distribution and elimination components (Table 1.2). Most previous studies have only demonstrated 2-compartment models, but few have sampled for the long duration that was examined in the present study.

The three compartments are presumed to represent: a central compartment comprising blood and highly-perfused tissues such as the liver, lungs and kidneys; a peripheral compartment comprising less well perfused tissues such as muscles and skin; and a deep tissue compartment comprising poorly-perfused tissues such as adipose tissue, cartilage, ligaments and bone (Gibaldi and Feldman, 1969). Alternatively, compartment 1 may simply represent absorption from the gut. In the absence of information, elimination is assumed to occur exclusively from the central compartment, and this is consistent with the observation that phenylbutazone appeared unchanged in sizeable concentrations in the urine. Nevertheless, some conversion of phenylbutazone to the metabolite OPB appears to have occurred in both plasma and urine.

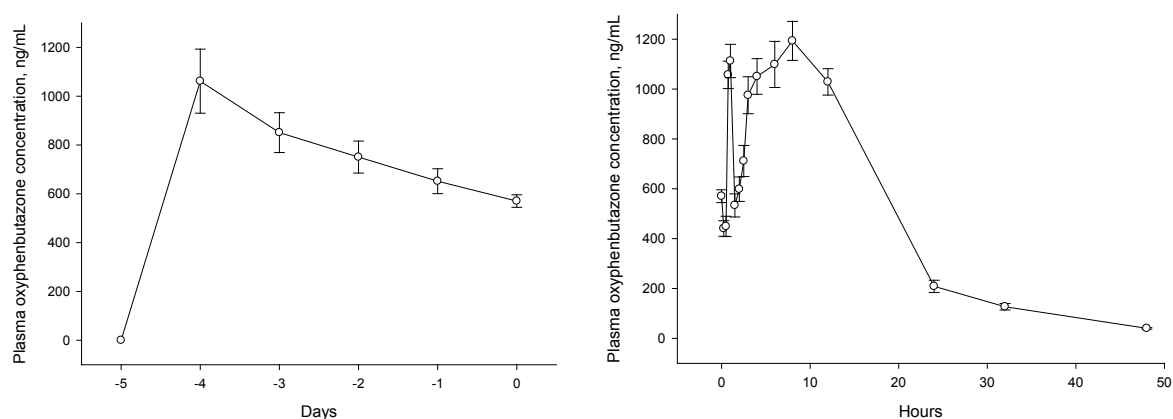


Figure 1.2 Plasma concentrations of oxyphenylbutazone (mean $\pm$ se) during and after a 5 day course of phenylbutazone administered orally to 12 horses commencing on day -5

Plasma concentrations of the metabolite OPB were lower than those of the parent drug during the treatment period (Table 1.1). After the last dose of phenylbutazone OPB concentrations showed a biphasic peak in plasma concentration up to 12 h, before declining slowly (Figure 1.2). The metabolite was still detected in all 12 horses after 4 days (96 h), in 5 of the 12 horses after 5 days (120 h), and in none of the horses after 6 days. The degree of variation in plasma phenylbutazone and OPB concentrations between horses was minor.

Table 1.2 Pharmacokinetic parameters for phenylbutazone in plasma during and following 5 days of oral dosing (2 g bid on day 1 then 1 g bid for 4.5 d) to 12 horses

	Mean $\pm$ SD	Min	Max	Median
Peak plasma concentration ($C_{\max}$), $\mu\text{g/L}$	13537 $\pm$ 2856	8462	17615	13365
Time to peak plasma concentration ($T_{\max}$), min	4.3 $\pm$ 1.7	2	8	4
Apparent volume of distribution (VT), L/kg	0.15 $\pm$ 0.04	0.10	0.24	0.15
Compartment 1 (absorption from gut)				
Terminal half-life ($T_{1/2}$ for α), h	1.55 $\pm$ 0.85	0.67	3.84	1.43
Compartment 2 (distribution)				
Terminal half-life ($T_{1/2}$ for β), h	5.6 $\pm$ 0.66	4.56	6.82	5.7
Compartment 3 (elimination)				
Terminal half-life ($T_{1/2}$ for γ), /h	37.3 $\pm$ 4.64	30.6	46.7	36.9

Urine phenylbutazone

Urine concentrations of phenylbutazone are shown in Table 1.1 and Figure 1.3, and urine OPB concentrations in Table 1.1 and Figure 1.4.

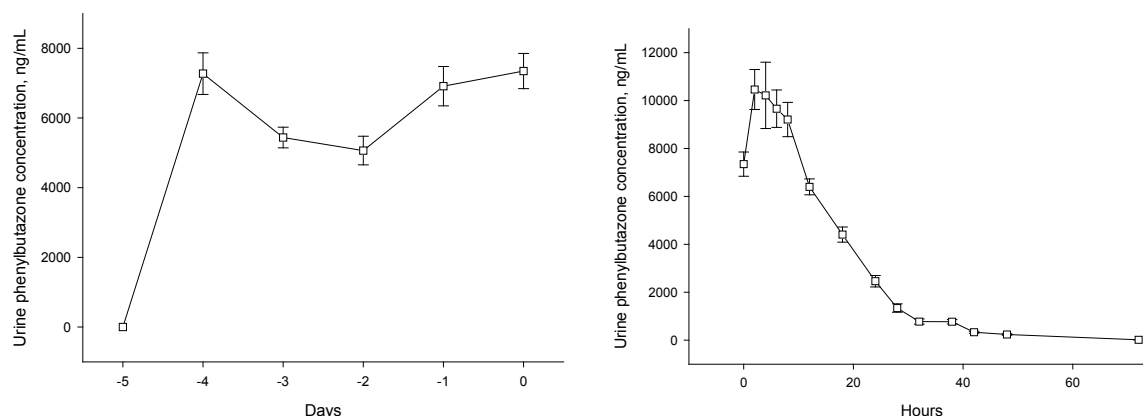


Figure 1.3 Urine concentrations of phenylbutazone (mean $\pm$ se) during and after a 5 day course of phenylbutazone administered orally to 12 horses commencing on day -5

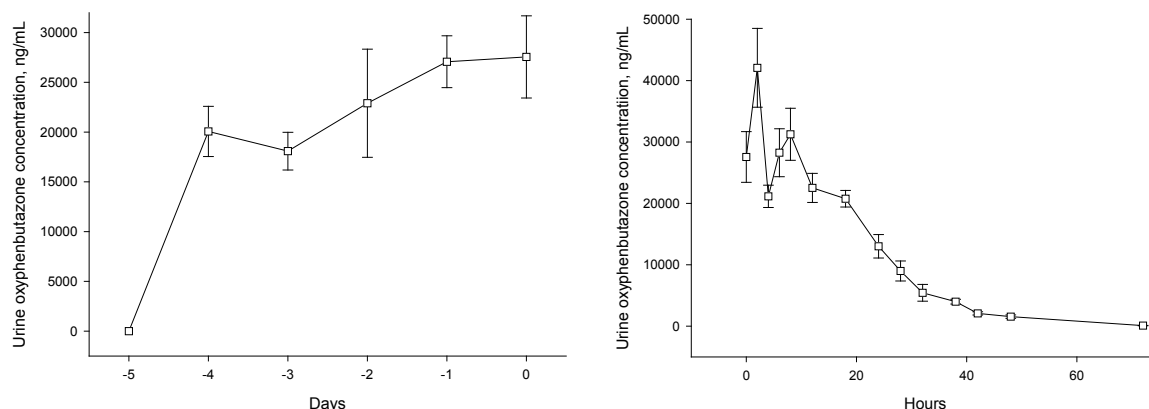


Figure 1.4 Oxyphenbutazone concentrations in urine (mean $\pm$ se) during and after a 5 day course of phenylbutazone administered orally to 12 horses commencing on day -5

Urine concentrations of both phenylbutazone and OPB were elevated during the treatment course (Fig. 1.3 and 1.4). Urine phenylbutazone concentrations were highest (Fig. 1.3; 10462 ng/mL) 2 h after the last dose of phenylbutazone and continued to decline until they were close to the limit of detection 9 days (216 h) after the last dose (Table 1.1).

Similarly, urine OPB concentration peaked 2 h after treatment (Fig. 1.4; 42077 $\pm$ 6417 ng/mL). However, they remained elevated for slightly longer than phenylbutazone and although OPB was largely excreted by 9 days, the metabolite was still readily detectable at this time point (Table 1.1).

Conclusions

This is the first study to examine the pharmacokinetics of phenylbutazone and OPB in plasma and urine in 12 horses over an extended sampling period. The pharmacokinetics of phenylbutazone fitted a 3-compartment model. The drug showed a characteristic twin peak in plasma between 2.5 and 4 h and it was 9 days before the drug was undetectable in the plasma of any horse. Phenylbutazone and OPB were also detectable in urine 9 days after administration. These data are currently being reviewed by the racing authorities to determine appropriate reporting levels.

2 Flunixin

Background

Flunixin is a non-steroidal, anti-inflammatory drug (NSAID) with a rapid onset and long duration of pharmacological action. It is reported to be more potent than other NSAIDs, such as phenylbutazone, but is non-narcotic (Beretta et al., 2005). Through inhibition of the COX enzyme pathway, the drug possesses anti-inflammatory, anti-pyretic and analgesic properties. It is used in the treatment of endotoxic shock, and pain and inflammation associated with musculoskeletal disorders, trauma and ocular conditions, such as uveitis (Moses and Bertone, 2002). Flunixin is also commonly used to manage visceral pain associated with colic.

The principle mode of drug delivery is by IV injection, and although the drug can be given intramuscularly (IM), it has been reported to be irritating to the tissues when given IM. Oral formulations are also available. Flunixin is given at a dose rate of 1.1 mg/kg once daily and is available in the following parenteral formulations: Finadyne Solution, Intervet/Schering-Plough Animal Health; Flumav, Mavlab; Ilium Flunixil, Troy Laboratories and Flunix, Bomac (eMIMS IVS Australia, 2011).

Flunixin is a carboxylic acid that is lipophilic and highly protein bound (Moses and Bertone, 2002). As with other NSAIDs, administration to horses with pre-existing renal, hepatic or cardiac compromise, gastric ulceration or haematological disorders is not recommended (Moses and Bertone, 2002; Radi and Khan, 2006).

Flunixin is rapidly distributed following IV injection and this is followed by a longer elimination phase. The plasma pharmacokinetics (PK) of flunixin have been described previously with a 2-compartment PK model observed following IV administration (Pellegrini-Masini et al., 2004). The aim of the current study was to measure both plasma and urine concentrations of flunixin meglumine following IV administration in a larger group (n = 12) of horses.

Flunixin administration

Flunixin (Flunix, Bomac) was administered IV (1.1 mg/kg BW) to 12 mature Standardbred geldings (mean body weight 476 ± 10.9 kg) housed at The University of Queensland, Gatton Campus, Qld.

Blood (40 mL) and urine (40 mL) samples were collected before the first dose of flunixin was administered and then at regular intervals of decreasing frequency following drug administration (Table 2.1).

Immediately after collection, blood samples were centrifuged (2,800 x g, 10 min). The plasma was divided into 2 portions and stored in plastic vials at -20°C . Urine samples were also divided into two portions and stored at -20°C . All samples were transported frozen to the Australian Racing Forensic Laboratories, NSW, where they were analysed for the presence of flunixin.

Extraction and analysis procedures

Flunixin- d_3 was used as an internal standard and was added to all urine and plasma samples before extraction. Urine samples were adjusted to basic pH by adding 1 mL of 1 M sodium hydroxide before being buffered to pH 3.2 with sodium hydrogen phosphate (2 mL) and extracted with dichloromethane (4 mL). The solutions were mixed for 10 min then centrifuged for 10 min at 2,800 x g. The organic layer was removed and evaporated to dryness under nitrogen at 60°C , and the residues were reconstituted with isopropanol and ammonium acetate.

Plasma samples were extracted after the addition of saturated sodium phosphate (pH 3, 2 mL) and HCl (10%, 4 to 6 drops). Extracts were evaporated to dryness then reconstituted with isopropanol (50 μ L) and pH 4 ammonium acetate (10 mM, 950 μ L).

Calibration curves for flunixin in urine and plasma were constructed over the range 0 to 16000 ng/mL using flunixin-d₃ as the internal standard. Quality control solutions contained flunixin at 50 ng/mL and 350 ng/mL (low), and 2000 ng/mL and 14000 ng/mL (high).

All reconstituted samples were analysed by LCMSMS using a Thermo Electron Corporation (San Jose, CA, USA) Finnigan TSQ Quantum Ultra instrument interfaced with a Surveyor LC pump, autosampler and degasser.

Pharmacokinetic modelling

The pharmacokinetics of ketoprofen and hydroxyketoprofen were modelled at Charles Sturt University, NSW and The University of Pennsylvania, PA, using WinSAAM software (<http://www.winsaam.com>, University of Pennsylvania) and STATA Statistical Software 11.1 (Stata, 2007) as described in the general methods section. PK data are presented as the mean $\pm$ SD. All other data are presented as mean $\pm$ se.

Results and discussion

Plasma flunixin

Table 2.1 Plasma and urine flunixin meglumine concentration (mean $\pm$ se) following intravenous dosing (1.1 mg/kg) in 12 horses

Time	Plasma (ng/mL)	Urine (ng/mL)
0 h	0	0
5 min	8728 $\pm$ 257	-
10 min	6994 $\pm$ 202	-
20 min	5686 $\pm$ 130	-
40 min	4422 $\pm$ 138	-
1 h	3515 $\pm$ 152	-
2 h	2176 $\pm$ 150	-
4 h	1019 $\pm$ 103	69285 $\pm$ 25464
8 h	238 $\pm$ 32.8	79503 $\pm$ 10107
12 h	87.9 $\pm$ 20.2	28497 $\pm$ 4311
16 h	13.8 $\pm$ 6.12	10048 $\pm$ 2545
24 h	2.67 $\pm$ 0.72	1754 $\pm$ 294
36 h	1.08 $\pm$ 0.19	184 $\pm$ 38.8
48 h	0.17 $\pm$ 0.11	61 $\pm$ 11.4
72 h	0.08 $\pm$ 0.08	22.2 $\pm$ 3.87
96 h	0.08 $\pm$ 0.08	16.2 $\pm$ 4.88
120 h	0	9.50 $\pm$ 1.40
144 h	0	6.90 $\pm$ 1.94
168 h	0	6.30 $\pm$ 1.91
192 h	0	6.40 $\pm$ 3.61
216 h	0	4.20 $\pm$ 1.32
240 h	0	4.10 $\pm$ 1.83

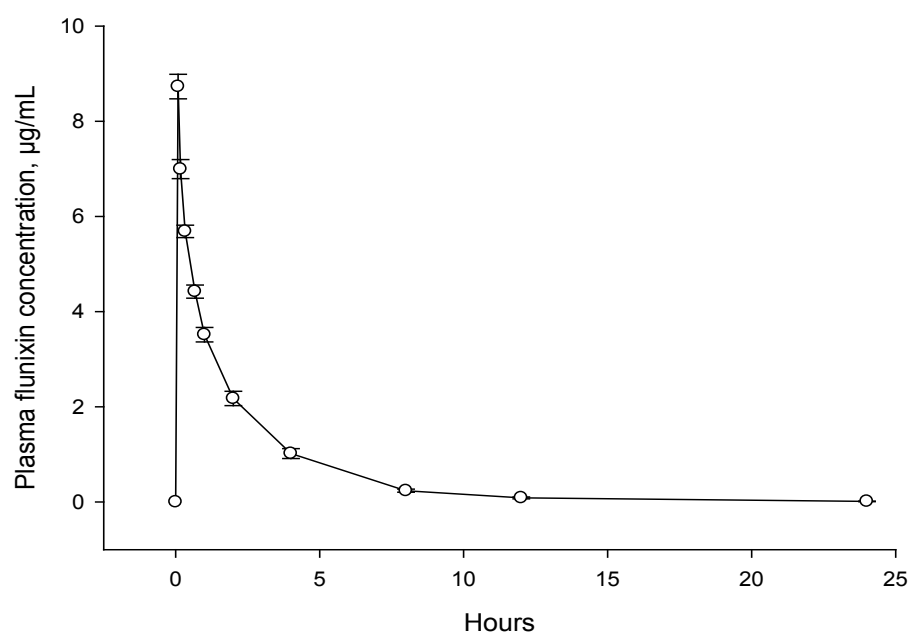


Figure 2.1 Plasma flunixin (mean $\pm$ se) concentrations following intravenous dosing (1.1 mg/kg) in 12 horses

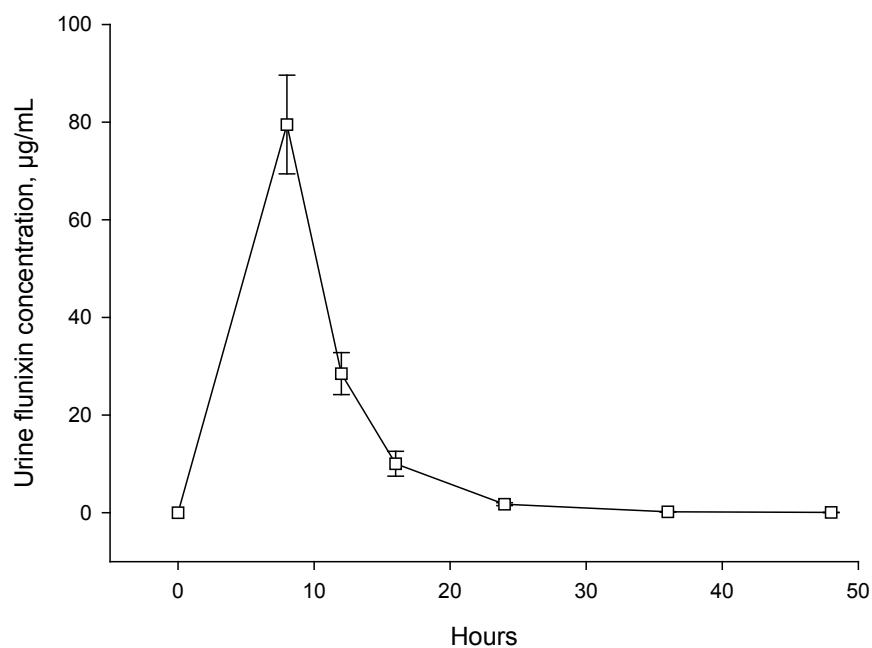


Figure 2.2 Urine flunixin concentrations (mean $\pm$ se) following intravenous dosing (1.1 mg/kg) in 12 horses

Plasma concentrations of flunixin are shown in Table 2.1 and Figure 2.1.

Flunixin reached a peak plasma concentration of 8728 ng/mL only 5 min after administration of the drug. The data fitted a two-compartment model (Table 2.2) with plasma half-lives of 1.53 h and 8.61 h

for each compartment, and most of the drug was cleared from the plasma within 12 h (Fig 2.1). However, flunixin was still detectable in 11 of the 12 horses 48 h after administration, in 2 horses at 72 h, and in 1 horse 96 h after treatment (Table 2.1). Overall, there was minimal variation in rate of drug metabolism between the horses.

Table 2.2 Pharmacokinetic parameters for flunixin meglumine following intravenous dosing (1.1 mg/kg) in 12 horses

	Mean $\pm$ SD	Min	Max	Median
Peak plasma concentration ($C_{\max}$), $\mu\text{g/L}$	8728 $\pm$ 892	7120	10395	8462
Time to peak plasma concentration ($T_{\max}$), min	5 $\pm$ 0	5	5	5
Apparent volume of distribution (V_T), L/kg	0.216 $\pm$ 0.022	0.184	0.25	0.212
Compartment 1				
Terminal half-life ($T_{1/2}$ for α), h	1.53 $\pm$ 0.25	1.14	1.95	1.58
Compartment 2				
Terminal half-life ($T_{1/2}$ for β), h	8.61 $\pm$ 3.93	4.27	18.49	7.95

Urine flunixin

Data for urine flunixin concentrations are shown in Table 2.1 and Fig. 2.2. Urine flunixin concentration peaked at 8 h following intravenous dosing (Table 2.1, 79503 ng/mL) and was largely eliminated within 48 h (Fig. 2.2). However, traces of flunixin were still detectable in the urine 240 h after treatment (Table 2.1).

Conclusions

Flunixin is an NSAID used in the treatment of pain and inflammation. Plasma concentrations peak within 5 min of IV administration and fall to low levels within 12 h. Nevertheless, flunixin is detectable in plasma for up to 96 h and in urine for up to 240 h after treatment.

These data are currently being reviewed by the racing authorities to determine appropriate reporting levels for the drug.

3 Ketoprofen

Background

Ketoprofen is a NSAID used to treat inflammation, pain and fever associated with musculoskeletal, ophthalmic and gastrointestinal disorders in the horse. It can also be used to manage post-operative and traumatic inflammation and pain. As with other NSAIDs, ketoprofen inhibits the COX enzyme pathway and is also reported to be a potential inhibitor of the lipo-oxygenase pathway, thus reducing inflammatory mediator release (Moses and Bertone, 2002).

Ketoprofen is of the carboxylic class of NSAIDs and has a good safety margin (MacAllister et al., 1993; Moses and Bertone, 2002; Mozaffari et al., 2010). However, its use has been associated with ulceration of the gastrointestinal mucosa in horses (MacAllister et al., 1993).

Formulations of the drug currently available in Australia include Ilium Ketoprofen injection (Troy Laboratories), Ketoprofen 10 Injectable (Merial) and Ketoprofen Injection (Nature Vet) (eMIMS IVS Australia, 2011). Ketoprofen is rapidly absorbed following slow IV or deep IM injection at a typical dose rate of 2.2 mg/kg once daily (Moses and Bertone, 2002). It is widely distributed throughout the body, including the synovial fluid, and is eliminated following metabolism by the liver to hydroxy-ketoprofen (Brink et al., 1998).

The pharmacokinetics of ketoprofen in plasma following IV dosing has been studied previously in horses, and fitted a 2-compartment model (Brink et al., 1998; Jaussaud et al., 1993). However, the sample size in these studies was smaller than in the current study. The aim of the current study was to determine the disposition and excretion of ketoprofen and its metabolite in plasma and urine following IV administration in 12 horses.

Ketoprofen administration

Ketoprofen (Ilium Ketoprofen Injection, Troy Laboratories) was administered IV as a single dose of 1 g/horse (10 mL of 100 mg/mL; equivalent to 1.86 - 2.29 mg/kg) to 12 mature Standardbred geldings (mean body weight 489 ± 11.6 kg) housed at The University of Queensland, Gatton Campus, Qld.

Blood (40 mL) and urine (40 mL) samples were collected before the first dose of ketoprofen was administered and then at regular intervals of decreasing frequency following drug administration (Table 3.1). Immediately after collection, blood samples were centrifuged (2, 800 x g, 10 min). The plasma was divided into 2 portions and stored in plastic vials at -20°C . Urine samples were also divided into two portions and stored at -20°C . All samples were transported frozen to the Racing Science Centre, Qld, where they were analysed for the presence of ketoprofen.

Extraction and analysis procedures

All samples were spiked with internal standards. Urine samples were also subjected to enzyme hydrolysis with β -glucuronidase/arylsulphatase prior to extraction. The pH of the urine and plasma samples was adjusted to 5.8 using HCl and/or ammonia and the samples were aliquoted into glass tubes. The extraction process involved the separation of both acidic and basic fractions and employed solid-phase extraction cartridges (UCT CSDAU503 500mg/3mL) containing mixed-mode packing material and mounted in an automated workstation. Acidic and basic analytes were fractionated and collected into separate tubes. All sample eluents were evaporated to dryness at 40°C under nitrogen and then reconstituted in acetonitrile/water (50:50 v/v) before analysis using LC-MS-MS.

Pharmacokinetic modelling

The pharmacokinetics of ketoprofen and hydroxyketoprofen were modelled at Charles Sturt University, NSW and The University of Pennsylvania, PA, using WinSAAM software (<http://www.winsaam.com>, University of Pennsylvania) and STATA Statistical Software 11.1 (Stata, 2007) as described in the general methods section. PK data are presented as the mean $\pm$ SD. All other data are presented as mean $\pm$ se.

Results and discussion

Plasma ketoprofen

Plasma concentrations of ketoprofen and its metabolite hydroxyketoprofen, are shown in Table 3.1 and Figures 3.1 and 3.2.

Table 3.1 Plasma and urine concentrations (mean $\pm$ se) of ketoprofen and hydroxyketoprofen prior to and following IV dosing with ketoprofen (1 g) in 12 horses

Time	Plasma ketoprofen (ng/mL)	Plasma hydroxy-ketoprofen (ng/mL)	Urine ketoprofen (ng/mL)	Urine hydroxy-ketoprofen (ng/mL)
0 h	0	0	0	0
5 min	10321 $\pm$ 808	674 $\pm$ 61.8	-	-
10 min	8351 $\pm$ 758	1405 $\pm$ 113	-	-
20 min	6126 $\pm$ 469	1876 $\pm$ 169	-	-
40 min	3653 $\pm$ 266	1512 $\pm$ 136	-	-
1 h	2170 $\pm$ 204	1007 $\pm$ 89.3	-	-
2 h	666 $\pm$ 54.9	312 $\pm$ 30.3	-	-
4 h	132 $\pm$ 16.3	48.3 $\pm$ 11.8	67740 $\pm$ 7798	60960 $\pm$ 7851
8 h	21.4 $\pm$ 2.55	6.88 $\pm$ 1.86	62696 $\pm$ 7498	57900 $\pm$ 7370
12 h	6.12 $\pm$ 0.59	1.59 $\pm$ 0.57	5404 $\pm$ 1806	4502 $\pm$ 1301
16 h	-	-	799 $\pm$ 225	548 $\pm$ 158
24 h	1.15 $\pm$ 0.21	0	295 $\pm$ 92.2	210 $\pm$ 54.8
28 h	-	-	115 $\pm$ 27.7	84.1 $\pm$ 12.2
32 h	-	-	60.9 $\pm$ 10.9	48.4 $\pm$ 5.6
36 h	0	0	32.4 $\pm$ 4.91	26.2 $\pm$ 3.29
40 h	-	-	40.6 $\pm$ 10.9	23.2 $\pm$ 2.68
48 h	0	0	34.1 $\pm$ 4.22	14.4 $\pm$ 2.24

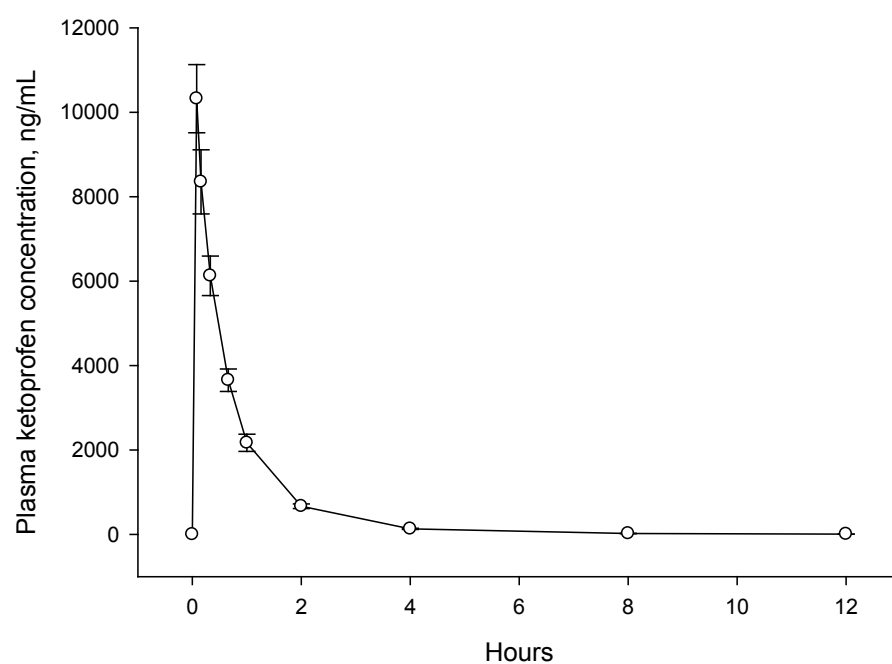


Figure 3.1 Plasma ketoprofen concentrations (mean $\pm$ se) after intravenous dosing (1 g) in 12 horses

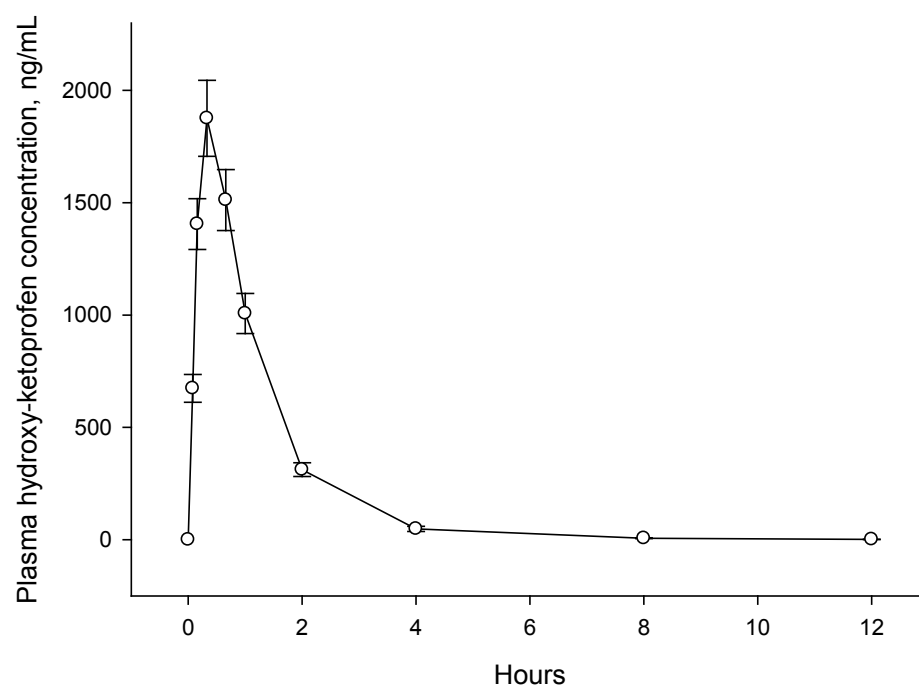


Figure 3.2 Plasma hydroxyketoprofen concentrations (mean $\pm$ se) after intravenous dosing (1 g) in 12 horses

Ketoprofen levels peaked in plasma (10321 ng/mL) 5 min after intravenous dosing and the drug was largely eliminated within 4 h (Fig 3.1). Nevertheless, ketoprofen was present at low concentrations in all horses 12 h after administration, becoming undetectable in all horses by 24 h.

The pharmacokinetics of ketoprofen fitted a 2-compartment model (Table 3.2), with half-lives of 36 min ($t_{1/2 \alpha}$) and 7.8 h ($t_{1/2 \beta}$).

Table 3.2 Pharmacokinetic parameters after intravenous dosing with ketoprofen (1 g) in 12 horses

	Mean $\pm$ SD	Min	Max	Median
Peak plasma concentration ($C_{\max}$), $\mu\text{g/L}$	10321 $\pm$ 2794	6360	13400	11500
Time to peak plasma concentration ($T_{\max}$), min	5 $\pm$ 0	5	5	5
Apparent volume of distribution (VT), L/kg	0.36 $\pm$ 0.18	0.19	0.73	0.27
Compartment 1				
Terminal half-life ($T_{1/2}$ for α), h	0.60 $\pm$ 0.12	0.47	0.84	0.56
Compartment 2				
Terminal half-life ($T_{1/2}$ for β), h	7.80 $\pm$ 5.08	3.91	19.8	6.12

Hydroxyketoprofen concentrations in plasma were slower to reach their peak (20 min), but also fell rapidly within 4 h of drug administration (Fig. 3.2). Hydroxyketoprofen concentrations in 4 horses showed a particularly rapid excretion time during the first phase and were only able to be fitted to a 2-compartment model. Data for the remaining 8 horses fitted a 3-compartment model, however, with apparent half-lives of 3.6 min, 43 min and 18 h (Table 3.3). This model should be interpreted with caution as some of the data points modelled during the third phase may have been below the limit of accurate quantification. In fact, mean hydroxyketoprofen concentrations were below the limit of accurate quantification within 8 h, and had fallen below this limit in all horses by 12 h.

Table 3.3 Pharmacokinetic parameters for the metabolite hydroxyketoprofen following the administration of ketoprofen (1 g) to 8* horses

	Mean $\pm$ SD	Min	Max	Median
Peak plasma concentration ($C_{\max}$), $\mu\text{g/L}$	1777 $\pm$ 349	1090	2216	1774
Time to peak plasma concentration ($T_{\max}$), min	20 $\pm$ 0	20	20	20
Apparent volume of distribution (VT), L/kg	1.73 $\pm$ 1.53	0.77	5.32	1.05
Compartment 1				
Terminal half-life ($T_{1/2}$ for α), h	0.06 $\pm$ 0.03	0.02	0.11	0.07
Compartment 2				
Terminal half-life ($T_{1/2}$ for β), h	0.72 $\pm$ 0.14	0.48	0.96	0.74
Compartment 3				
Terminal half-life ($T_{1/2}$ for γ), /h	18.3 $\pm$ 13.2	2.93	46.9	17.2

*Data for another four horses showed a brief excretion time and were only able to be fitted by a 2-compartment model

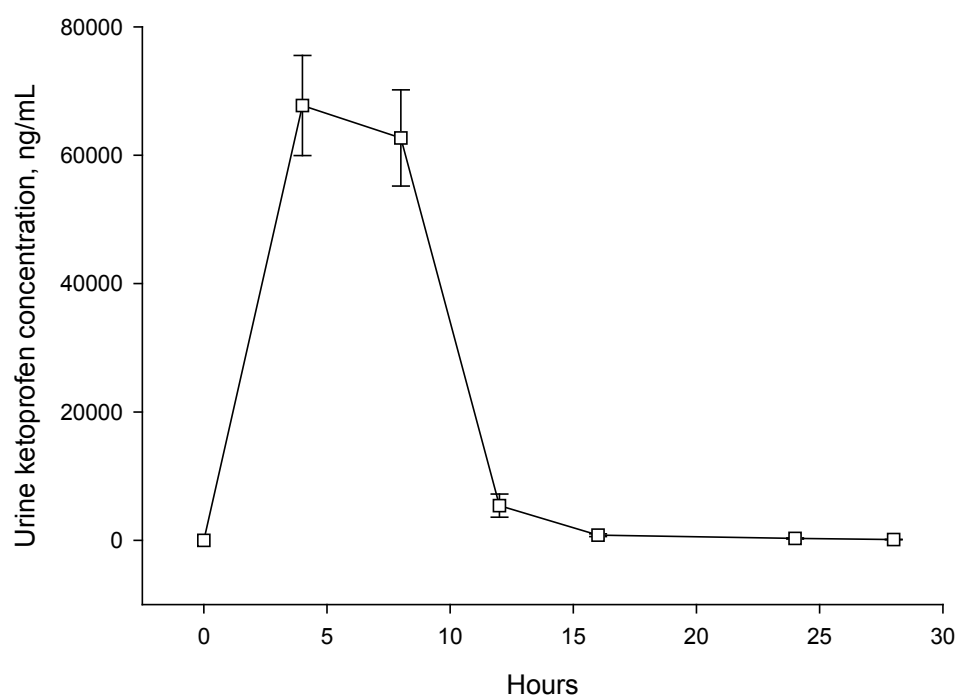


Figure 3.3 Urine ketoprofen concentrations (mean $\pm$ se) after intravenous dosing (1 g) in 12 horses

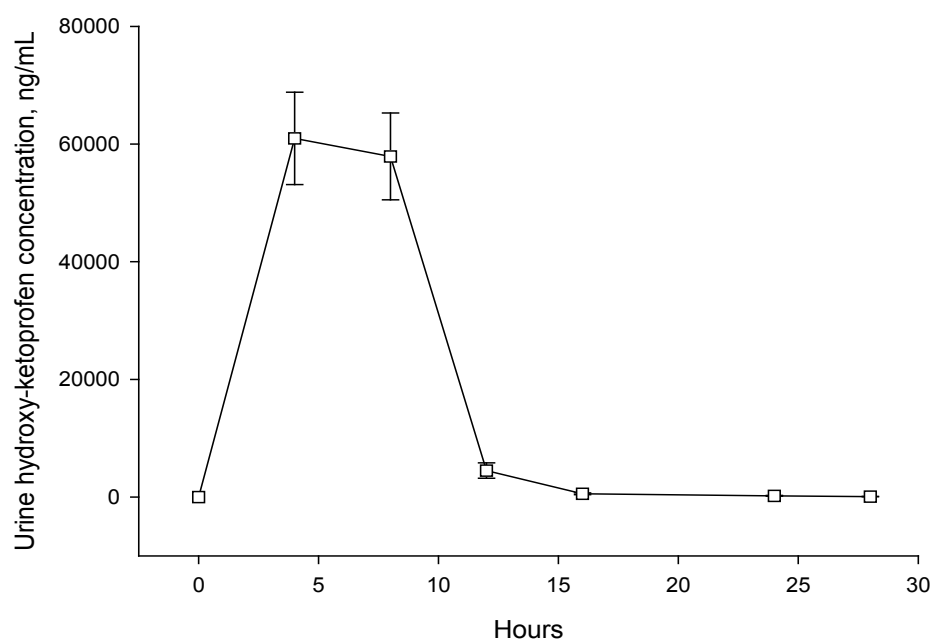


Figure 3.4 Urine hydroxyketoprofen concentrations (mean $\pm$ se) after intravenous dosing (1 g) in 12 horses

Urine ketoprofen

Urine concentrations of ketoprofen and hydroxyketoprofen are shown in Table 3.1 and in Fig. 3.3 and 3.4. Urinary excretion rates of ketoprofen and hydroxyketoprofen were similar. Both the parent drug and its metabolite remained at high concentrations in the urine for 8 h following dosing (Figs 3.3 and 3.4).

Ketoprofen was detectable in all 12 horses 48 h after dosing, but had fallen below the limit of quantification in all horses by 72 h. Hydroxyketoprofen was only detectable in 2 horses 48 h after ketoprofen administration and was also undetectable in all horses by 72 h.

Conclusions

This study has shown that following IV administration, ketoprofen undergoes a rapid phase of metabolism, becoming largely eliminated from plasma within 4 h. This is followed by a second, slower phase of metabolism, with the result that ketoprofen is detectable for at least 24 h in plasma and 48 h in urine. Ketoprofen becomes undetectable in plasma by 24 h and undetectable in urine by 72 h. These data are currently being examined by the horseracing authorities with a view to deciding appropriate reporting levels for the drug.

4 Hydrocortisone

Background

Hydrocortisone is a naturally-occurring corticosteroid which is used in veterinary medicine as an anti-inflammatory agent. It is often administered by IV or IM injection in the form hydrocortisone hemisuccinate which is highly water-soluble and allows high blood levels of hydrocortisone to be attained rapidly. Parenteral preparations of hydrocortisone available in Australia include Solu-Cortef (Pfizer Animal Health). It is also commonly available in a variety of topical preparations that contain hydrocortisone acetate, including eye ointments (Chloroptone, Delta Laboratories) and skin creams (Ilium Neocort, Troy Laboratories). Transdermal absorption of hydrocortisone into the systemic circulation can occur following topical application (Mills and Cross, 2006).

The rules of racing prohibit a horse from competing if an endogenous substance such as hydrocortisone is present in the animal on race day at an unnaturally high concentration. In practice, the use of hydrocortisone has been controlled by means of a threshold concentration in urine (Popot et al., 1997). However, normal endogenous hydrocortisone production shows a marked diurnal variation, as well as variations due to numerous stimuli such as excitement, stress and metabolic status. Therefore, it has been necessary to set the threshold beyond which hydrocortisone concentrations are viewed as abnormal, at the relatively high level of 1,000 ng/mL of urine.

Anecdotally, there is evidence that this threshold may be too high to adequately control the inappropriate use of hydrocortisone and that use of the injectable preparation on race day, has become widespread.

Others have studied of hydrocortisone concentrations in equine blood and urine. Studies based on immunoassay measurements, and the relative merits of immunoassays compared with more specific methods based on HPLC, have been reviewed by Houghton and Ginn (1992). There have also been studies of the metabolism of hydrocortisone in the horse (Popot et al., 1994, 1999), but no studies have examined the pharmacokinetics of hydrocortisone in a large group of horses. Further details of the present study have been reported by Vine et al. (2008).

Hydrocortisone administration

Hydrocortisone sodium succinate (1g Solu-Cortef® in 8 mL of vehicle @ 125 mg/mL) was administered by IV injection to 10 mature geldings (5 Thoroughbred, 5 Standardbred, mean body weight 545 ± 14 kg) housed at Charles Sturt University, NSW.

Blood and urine samples were collected three times daily (morning, afternoon and evening) for a week before hydrocortisone administration, to establish baseline levels of endogenous hydrocortisone. Following hydrocortisone injection, blood samples were collected at 0 min, 15 min, 30 min, 45min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h and 12 h, while urine samples were collected at 0 min, 1 h, 2 h, 3 h, 6 h, 8 h and 12 h, then twice daily for 7 days (Table 4.1).

Immediately after collection, blood samples were centrifuged (2,800 x g, 20 min). The plasma was divided into 2 portions and stored in plastic vials at -20°C . Urine samples were also divided into two portions and stored at -20°C . All samples were transported frozen to Racing Analytical Services, Vic., where they were analysed for the presence of hydrocortisone and its metabolites.

Extraction and analysis procedures

The analytical methodology used in this study was aligned with that used in the parallel studies of other drugs being coordinated by the European Horseracing Scientific Liaison Committee. In this way the two studies would be complementary and the data exchangeable, thereby enhancing the benefit to the international racing community. Details of this methodology have been published by Vine et al. (2008).

Initial validation studies were carried out for hydrocortisone hemisuccinate, hydrocortisone, its metabolic precursors and its metabolites with and without enzyme hydrolysis, using liquid/liquid and solid phase extraction methods with hydrocortisone-d₄ and triamcinolone acetonide as internal standards. Analysis was carried out by LC-MS-MS. Precision and accuracy were determined by intra- and inter-day analysis of control materials.

Calibration materials for the quantification of hydrocortisone and hydrocortisone hemisuccinate in plasma were prepared in synthetic plasma as described by Vine et al. (2006). Total hydrocortisone levels were determined in urine samples after enzyme hydrolysis with β -glucuronidase and solid phase extraction using Bond-Elut C18 columns. Plasma samples were processed by adding acetonitrile and vortexing prior to centrifugation. The supernatant was evaporated to dryness and reconstituted in acetic acid/methanol. All samples were analysed by LC-MS.

Pharmacokinetic modelling

The pharmacokinetics of hydrocortisone and hydrocortisone hemisuccinate in plasma were modelled at Charles Sturt University, NSW and The University of Pennsylvania, PA, using WinSAAM software (<http://www.winsaam.com>, University of Pennsylvania) and STATA Statistical Software 11.1 (Stata, 2007) as described in the general methods section. PK data are presented as the mean $\pm$ SD. All other data are presented as mean $\pm$ se.

Results and discussion

Plasma hydrocortisone

Figure 4.1 illustrates the diurnal variation in hydrocortisone concentrations before administration.

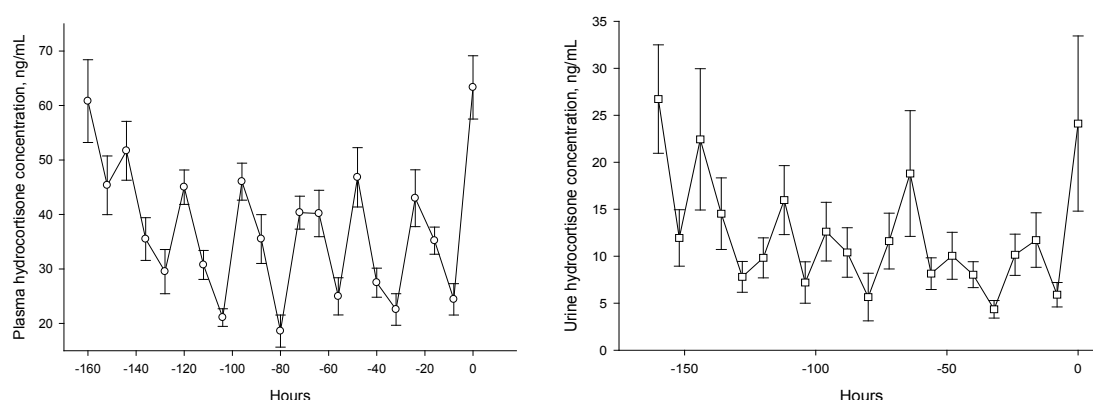


Figure 4.1 Endogenous hydrocortisone concentrations (mean $\pm$ se) in the plasma and urine of 10 horses prior to dosing with intravenous hydrocortisone

Table 4.1 Concentrations of hydrocortisone and hydrocortisone hemisuccinate (mean $\pm$ se) in plasma and urine prior to and following IV dosing with hydrocortisone hemisuccinate (1 g) in 10 horses

Time	Plasma hydrocortisone (ng/mL)	Plasma HC hemisuccinate (ng/mL)	Urine hydrocortisone (ng/mL)	Urine HC hemisuccinate (ng/mL)
-160	60.8 $\pm$ 7.60	-	26.7 $\pm$ 5.77	-
-152	45.4 $\pm$ 5.39	-	11.9 $\pm$ 3.00	-
-144	51.7 $\pm$ 5.40	-	22.4 $\pm$ 7.52	-
-136	35.5 $\pm$ 3.92	-	14.5 $\pm$ 3.81	-
-128	29.5 $\pm$ 4.05	-	7.81 $\pm$ 1.64	-
-120	45.0 $\pm$ 3.16	-	9.83 $\pm$ 2.13	-
-112	30.7 $\pm$ 2.66	-	16.0 $\pm$ 3.67	-
-104	21.1 $\pm$ 1.62	-	7.21 $\pm$ 2.20	-
-96	46.0 $\pm$ 3.40	-	12.6 $\pm$ 3.12	-
-88	35.5 $\pm$ 4.47	-	10.4 $\pm$ 2.63	-
-80	18.6 $\pm$ 2.94	-	5.66 $\pm$ 2.53	-
-72	40.3 $\pm$ 3.02	-	11.6 $\pm$ 2.97	-
-64	40.2 $\pm$ 4.27	-	18.8 $\pm$ 6.69	-
-56	25.0 $\pm$ 3.41	-	8.15 $\pm$ 1.68	-
-48	46.8 $\pm$ 5.45	-	10.0 $\pm$ 2.50	-
-40	27.5 $\pm$ 2.66	-	8.04 $\pm$ 1.38	-
-32	22.6 $\pm$ 2.90	-	4.36 $\pm$ 0.93	-
-24	43.0 $\pm$ 5.22	-	10.2 $\pm$ 2.19	-
-16	35.2 $\pm$ 2.49	-	11.7 $\pm$ 2.9	-
-8	24.4 $\pm$ 2.87	-	5.91 $\pm$ 1.3	-
0	63.3 $\pm$ 5.81	0	24.1 $\pm$ 9.32	0
0.25	7438 $\pm$ 1794	19786 $\pm$ 8292	-	-
0.5	4037 $\pm$ 942	9224 $\pm$ 3318	-	-
0.75	1250 $\pm$ 77.5	1786 $\pm$ 397	-	-
1	1157 $\pm$ 229	2095 $\pm$ 963	36252 $\pm$ 6734	52387 $\pm$ 15326
2	644 $\pm$ 88.9	1058 $\pm$ 294	14427 $\pm$ 2265	7197 $\pm$ 5089
3	431 $\pm$ 79.4	764 $\pm$ 379	-	-
4	292 $\pm$ 44.6	577 $\pm$ 205	4909 $\pm$ 777	778 $\pm$ 331
6	219 $\pm$ 55.4	480 $\pm$ 218	1765 $\pm$ 465	170 $\pm$ 47.5
8	220 $\pm$ 69.6	562 $\pm$ 260	339 $\pm$ 55.7	48.3 $\pm$ 8.20
12	109 $\pm$ 45.9	283 $\pm$ 192	25.2 $\pm$ 5.67	5.61 $\pm$ 1.54
24	21.7 $\pm$ 3.61	-	14.3 $\pm$ 4.73	2.94 $\pm$ 1.82
36	17.0 $\pm$ 2.61	-	9.15 $\pm$ 3.06	-
48	44.0 $\pm$ 5.16	-	23.3 $\pm$ 7.31	-
60	26.2 $\pm$ 3.70	-	19.4 $\pm$ 5.79	-
72	44.3 $\pm$ 4.18	-	30.5 $\pm$ 8.54	-
84	24.5 $\pm$ 2.33	-	16.1 $\pm$ 2.03	-
96	41.1 $\pm$ 5.57	-	30.0 $\pm$ 6.23	-

108	31.4 ± 3.80	-	24.0 ± 5.76	-
120	42.1 ± 3.35	-	42.3 ± 9.83	-
132	27.6 ± 3.77	-	17.2 ± 3.26	-
144	45.3 ± 5.25	-	41.6 ± 12.3	-
156	31.5 ± 3.39	-	16.6 ± 2.18	-
168	45.3 ± 5.22	-	35.6 ± 8.58	-
180	31.1 ± 3.45	-	25.4 ± 6.27	-

Concentrations of hydrocortisone and hydrocortisone hemisuccinate, before and after dosing with hydrocortisone hemisuccinate, are shown in Table 4.1. Although the daily variation was significant, mean concentrations of hydrocortisone in urine never exceeded 27 ng/mL and the highest value recorded in any individual horse on any day was 104 ng/mL, which is almost 10 times below the current threshold for forensic testing of 1,000 ng/mL.

Figures 4.2 and 4.3 show plasma concentrations of hydrocortisone and hydrocortisone hemisuccinate. The PK parameters for these compounds are shown in Tables 4.2 and 4.3. According to the modelled data, hydrocortisone concentrations peaked at 7883 ng/mL after 18 min and decreased rapidly for the first 45 min, then more gradually for the next 12 h, falling within the normal range by 24 h. The elimination pattern followed a 2-compartment PK model, with half-lives of 40.2 min ($t_{1/2\alpha}$) and 10 h 8 min ($t_{1/2\beta}$) for each compartment.

The administration of exogenous corticosteroids usually causes a marked suppression of endogenous corticosteroid production for several days, due to the activation of negative feedback mechanisms in the hypothalamus and a decrease in ACTH secretion. This response is very reliable and is exploited in some diagnostic procedures such as the dexamethasone suppression test which is used to identify horses with pituitary *pars-intermedia* dysfunction (PPID), or ‘equine Cushing’s disease’ (McFarlane, 2011). In the present study, for the period 1.5 to 4.5 days after hydrocortisone hemisuccinate administration, endogenous hydrocortisone concentrations averaged 31.3 ng/mL, compared with 50 ng/mL during the pre-administrations phase. Although this suggests some degree of adrenal suppression, the marked suppression that is normally expected, was not seen.

A second unexpected finding was that hydrocortisone hemisuccinate itself was detected in both plasma and urine, and at high concentrations, estimated to peak at 21,626 ng/mL in plasma within 25 min of administration (according to the PK model, Table 4.3) and at 52,387 ng/mL in urine within 1 h (the time at which the first post-treatment samples were collected). As with hydrocortisone, the elimination pattern of hydrocortisone hemisuccinate followed a 2-compartment PK model, with half-lives of 12 min ($t_{1/2\alpha}$) and 2 h 17 min ($t_{1/2\beta}$) for each compartment.

This facile detection of the parent compound was not anticipated, as its conversion to hydrocortisone *in vivo* was expected to be too rapid. The slower conversion rate observed suggests that either the hydrolysis of the hemisuccinate ester is not particularly rapid, or that the large dose (1 g) had temporarily overwhelmed the hydrolytic process in these horses.

The fact that hydrocortisone hemisuccinate was measurable in plasma in all 10 horses for up to 12 h, and in urine in some horses for up to 24 h after administration, is an important observation, as it provides an opportunity for tighter control over the use of this substance, especially when it is considered that urinary hydrocortisone exceeded the current threshold level for reporting for only 6 h and there is currently no threshold for plasma hydrocortisone.

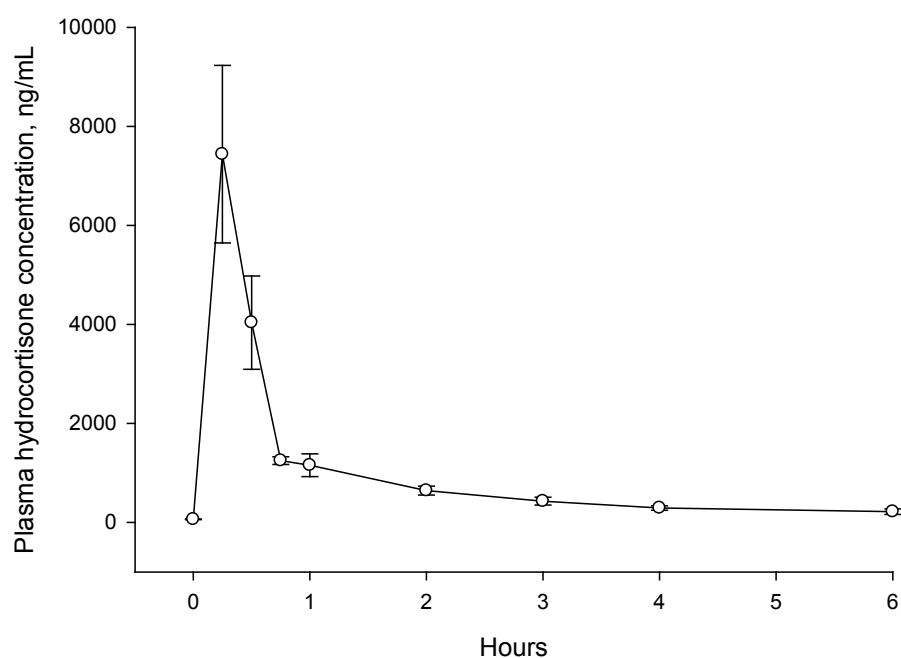


Figure 4.2 Plasma concentrations of hydrocortisone (mean $\pm$ se) following intravenous dosing with hydrocortisone hemisuccinate (1 g) in 10 horses

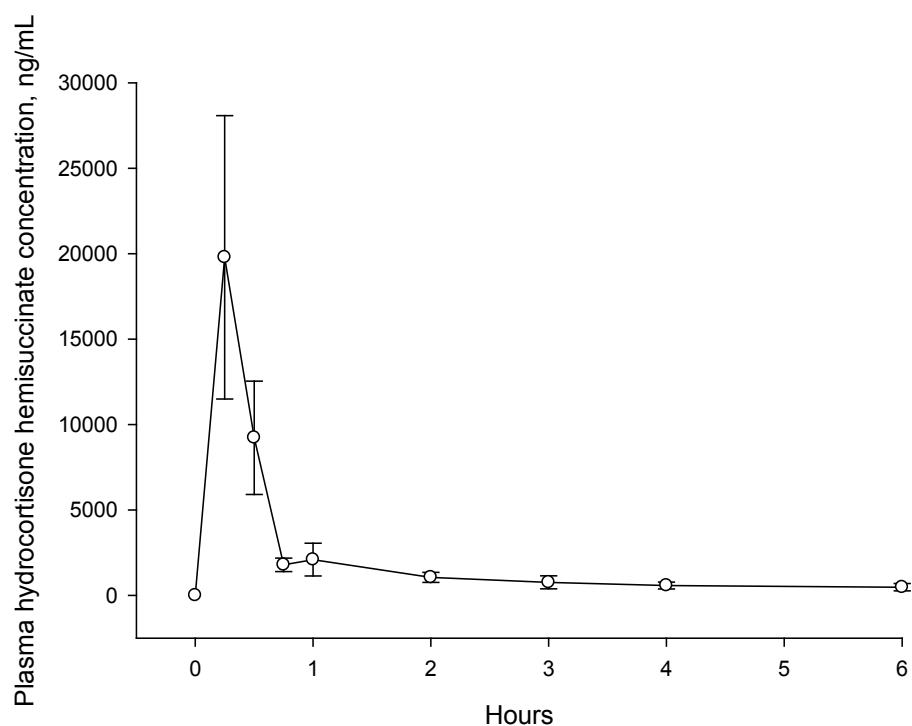


Figure 4.3 Plasma concentrations of hydrocortisone hemisuccinate (mean $\pm$ se) following intravenous dosing (1 g) in 10 horses

Table 4.2 Pharmacokinetic parameters for hydrocortisone in plasma following the administration of hydrocortisone hemisuccinate (1 g) to 10 horses

	Mean $\pm$ SD	Min	Max	Median
Peak plasma concentration ($C_{\max}$), $\mu\text{g/L}$	7883 $\pm$ 5502	3611	22292	6659
Time to peak plasma concentration ($T_{\max}$), h	18 $\pm$ 6.3	15	30	15
Apparent volume of distribution (V), L/kg	0.60 $\pm$ 0.38	0.015	1.21	0.67
Compartment 1				
Terminal half-life ($T_{1/2}$ for α), h	0.67 $\pm$ 0.40	0.09	1.27	0.67
Compartment 2				
Terminal half-life ($T_{1/2}$ for β), h	10.1 $\pm$ 5.51	1.96	19.6	11.2

Table 4.3 Pharmacokinetic parameters for hydrocortisone hemisuccinate in plasma following intravenous dosing (1 g) in 10 horses

	Mean $\pm$ SD	Min	Max	Median
Peak plasma concentration ($C_{\max}$), $\mu\text{g/L}$	21626 $\pm$ 26022	2688	90520	16345
Time to peak plasma concentration ($T_{\max}$), min	18 $\pm$ 6.3	15	30	15
Apparent volume of distribution (V), L/kg	0.11 $\pm$ 0.1	0.002	0.32	0.09
Compartment 1				
Terminal half-life ($T_{1/2}$ for α), h	0.2 $\pm$ 0.16	0.06	0.56	0.15
Compartment 2				
Terminal half-life ($T_{1/2}$ for β), /h	2.29 $\pm$ 1.47	0.68	6.16	1.95

Urine hydrocortisone

Concentrations of hydrocortisone and hydrocortisone hemisuccinate in urine are shown in Figures 4.4 and 4.5.

The hydrocortisone hemisuccinate concentrations are of a similar order as the respective hydrocortisone concentrations and it is clear from the excretion data that the concentration of both substances in urine drops very rapidly.

At 4 h post-administration all 10 horses had urinary hydrocortisone concentrations above the 1,000 ng/mL reporting threshold used to define whether a sample is “positive” or not. However, at 6 h, four horses were below the threshold. Therefore, the current international threshold can only provide evidence of the use of a 1g dose of hydrocortisone hemisuccinate for 4 to 6 h after dosing, and this is consistent with the widely held view that the threshold lacks sensitivity.

In contrast, hydrocortisone hemisuccinate could be detected in all 10 horses at 8 h, and in 7 out of 10 horses at 12 h. Thus, if detection is forced to rely on urine analysis, analysis of the hemisuccinate ester not only provides a longer detection time, but avoids any dispute over whether the analyte is of endogenous or exogenous origin.

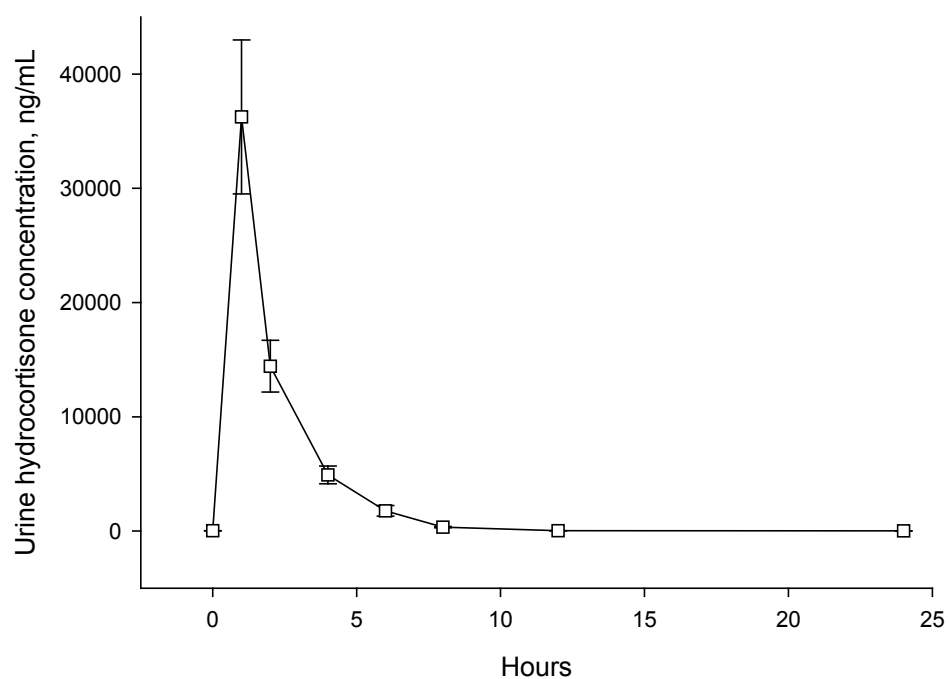


Figure 4.4 Urine concentrations of hydrocortisone (mean $\pm$ se) following intravenous dosing with hydrocortisone hemisuccinate (1 g) in 10 horses

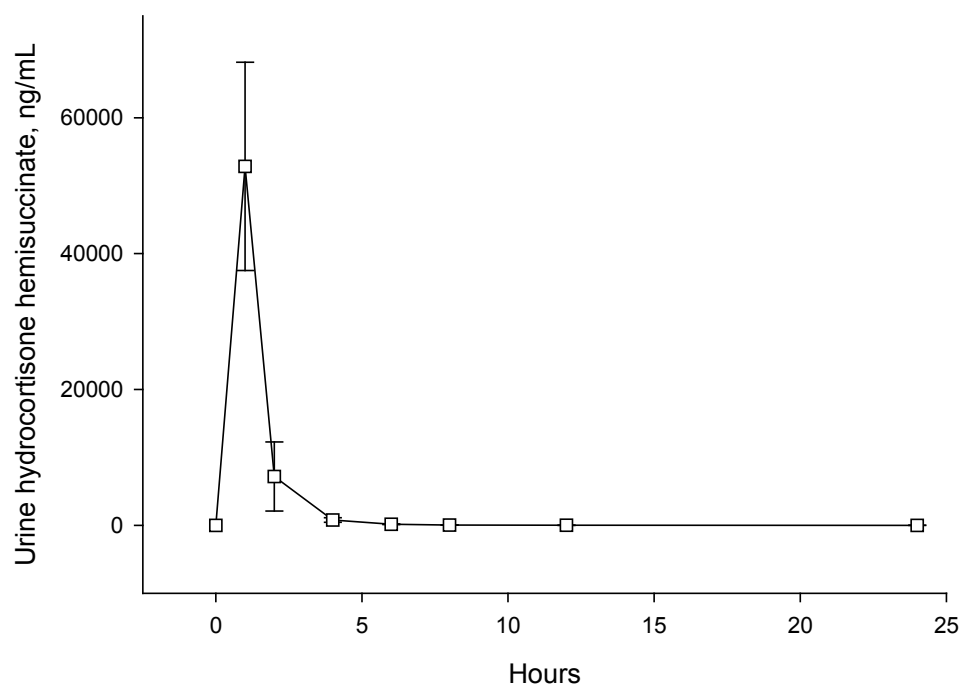


Figure 4.5 Urine concentrations of hydrocortisone hemisuccinate (mean $\pm$ se) following intravenous dosing (1 g) in 10 horses

Conclusions

This is the first detailed study of hydrocortisone metabolism conducted in a large number of horses.

The elimination of exogenous hydrocortisone from the body was found to follow a two-phase process, with the slower phase exhibiting a half-life of just over 10 hours. Nevertheless, concentrations of hydrocortisone in urine exceeded the current threshold for reporting of 1,000 ng/mL for only 6 h after administration. Furthermore, the marked suppression of endogenous hydrocortisone production which is normally seen after the administration of synthetic corticosteroids was not observed in the present study, pointing to the limitations of relying on adrenal suppression as a forensic marker of corticosteroid use.

The results also showed that after hydrocortisone hemisuccinate administration the parent compound is not converted hydrocortisone as rapidly as expected, and that the hemisuccinate can be detected in plasma and urine for up to 24 h in some cases. This observation provides forensic laboratories with an opportunity to exert tighter control over the illicit use of injectable substances such as Solu-Cortef[®] on race day.

5 Prednisolone

Background

Prednisolone is a corticosteroid derived from hydrocortisone. It has approximately 3 to 5 times the glucocorticoid and anti-inflammatory activity of hydrocortisone. Thus, it is used more frequently than hydrocortisone as an anti-inflammatory and anti-allergy drug in veterinary medicine (eMIMS IVS Australia, 2011).

Intravenous administration of 50 to 100 mg of prednisolone sodium succinate (Solu-delta-cortef solution, Pfizer Animal Health) is used in horses to treat acute (or peracute) endotoxic or traumatic shock and acute hypersensitivity reactions. Oral prednisolone (Macrolone granules, Mavlab and Predgy granules, Vetsearch International) is administered to horses to alleviate allergic skin conditions, chronic inflammation and non-infectious respiratory disease (Leclerc et al., 2010). Oral dose rates in horses depend on the type and severity of the condition being treated, but typically commence at 1 mg/kg and are then reduced to maintenance levels (and/or alternate day therapy) after an initial 3 to 5 days of therapy. Dose rates are lower in ponies.

Prednisolone is highly bound to plasma proteins in the horse (Alvinerie et al., 1988). The administration of prednisolone is contra-indicated in the presence of laminitis, corneal ulceration and renal disease (Johnson et al., 2002). Corticosteroids can also delay wound healing and fracture repair times. Prednisolone use is not recommended in the presence of concurrent equine Cushing's disease, renal disease or uncontrolled infections.

Prednisolone is metabolised to prednisone, dihydroprednisolone and dihydroprednisone. There are minimal data on the pharmacokinetics of prednisolone following oral dosing in the horse, although PK studies have been performed following IV and IM routes of administration (Chen et al., 1995; Toutain et al., 1984). The aim of the current study was to explore the pharmacokinetics of prednisolone and its metabolites in plasma and urine following 5 days of oral dosing in 12 horses.

Prednisolone administration

Prednisolone (Predgy granules, Vetsearch International) was administered orally (1 g/day for 5 consecutive days) to 12 mature Standardbred geldings (mean body weight 504 ± 13.7 kg) housed at The University of Queensland, Gatton, Qld.

Blood and urine samples were collected once prior to the first dose of prednisolone, then once daily on the final 3 days of prednisolone treatment. Following the final oral dose of prednisolone blood and urine samples were collected at regular intervals of decreasing frequency for up to 36 h post dosing for blood samples, and 96 h for urine samples (Tables 5.1 and 5.6).

Immediately after collection, blood (40 mL) and urine (40 mL) samples were centrifuged (2, 800 x g, 10 min). The plasma was divided into 2 portions and stored in plastic vials at -20°C . Urine samples were also divided into two portions and stored at -20°C . All samples were transported frozen to the Australian Racing Forensic Laboratories, NSW, where they were analysed for the presence of prednisolone and its metabolites.

Extraction and analysis procedures

All samples were spiked with an internal standard prior to extraction. Urine samples were also subjected to enzyme hydrolysis using β -glucuronidase (*E. coli* type IX-A). Plasma and hydrolysed

urine samples were extracted using an automated extraction method employing solid-phase extraction cartridges (UCT CEC18 500mg/3mL). Sample extracts were washed with a dilute caustic solution then evaporated to dryness under dry nitrogen. The extracts were then reconstituted in acetonitrile/water (50:50 v/v) and analysed using LC-MS-MS.

Pharmacokinetic modelling

The pharmacokinetics of prednisolone and its metabolites in plasma were modelled at Charles Sturt University, NSW and The University of Pennsylvania, PA, using STATA Statistical Software 11.1 (Stata 2007) as described in the general methods section. PK data are presented as the mean $\pm$ SD. All other data are presented as mean $\pm$ se.

Results and discussion

Plasma prednisolone

Concentrations of prednisolone and three of its metabolites: prednisone, dihydroprednisolone and dihydroprednisone, are shown in Table 5.1 and Figure 5.1.

Table 5.1 Plasma concentrations of prednisolone and three metabolites (mean $\pm$ se) following oral dosing with prednisolone (1 g daily for 5 d) in 12 horses

Time	Prednisolone (ng/mL)	Prednisone (ng/mL)	Dihydroprednisolone (ng/mL)	Dihydroprednisone (ng/mL)
0h	0	0	0	0
15 min	257 $\pm$ 29.2	8.55 $\pm$ 1.01	206 $\pm$ 97.5	4.52 $\pm$ 0.91
30 min	411 $\pm$ 36.4	15.9 $\pm$ 1.97	562 $\pm$ 167	15.2 $\pm$ 1.85
45 min	369 $\pm$ 45.4	18.7 $\pm$ 2.51	766 $\pm$ 397	21.2 $\pm$ 2.58
1 h	287 $\pm$ 35.5	18.4 $\pm$ 2.19	660 $\pm$ 264	29.5 $\pm$ 3.32
1 h 15 min	254 $\pm$ 22.3	18.6 $\pm$ 2.22	689 $\pm$ 232	37.8 $\pm$ 5.12
1 h 30 min	188 $\pm$ 15.4	13.8 $\pm$ 1.22	523 $\pm$ 195	35.2 $\pm$ 4.46
1 h 45 min	166 $\pm$ 20.6	15.0 $\pm$ 2.40	464 $\pm$ 146	37.7 $\pm$ 4.71
2 h	147 $\pm$ 15.0	15.8 $\pm$ 1.99	525 $\pm$ 142	45.3 $\pm$ 4.51
2 h 30 min	120 $\pm$ 15.7	13.6 $\pm$ 2.15	438 $\pm$ 197	53.4 $\pm$ 7.40
3 h	96.5 $\pm$ 9.88	11.9 $\pm$ 1.55	329 $\pm$ 120	53.1 $\pm$ 8.03
3 h 30 min	78.8 $\pm$ 11.0	10.1 $\pm$ 1.48	286 $\pm$ 118	54.0 $\pm$ 7.40
4 h	51.3 $\pm$ 4.68	6.45 $\pm$ 0.77	177 $\pm$ 53.8	38.3 $\pm$ 3.93
5 h	46.6 $\pm$ 4.31	5.00 $\pm$ 0.67	168 $\pm$ 90.9	42.5 $\pm$ 6.36
6 h	35.7 $\pm$ 2.39	3.94 $\pm$ 0.46	121 $\pm$ 30.3	34.5 $\pm$ 2.56
7 h	24.4 $\pm$ 2.35	2.87 $\pm$ 0.60	87.9 $\pm$ 31.1	26.9 $\pm$ 2.95
8 h	13.6 $\pm$ 1.69	1.49 $\pm$ 0.51	53.0 $\pm$ 19.8	16.7 $\pm$ 1.99
12 h	1.33 $\pm$ 0.70	0	12.2 $\pm$ 9.84	4.35 $\pm$ 1.05
24 h	0	0	0	0
36 h	0	0	-	-

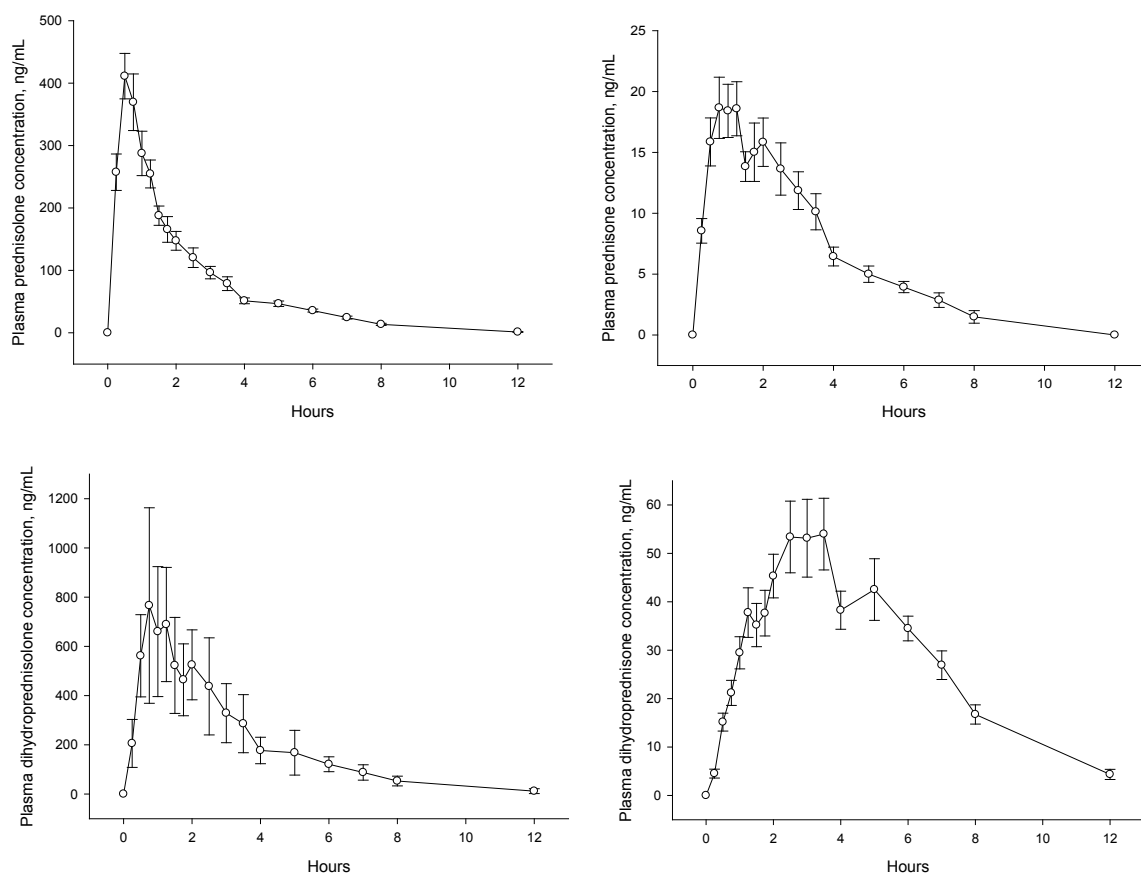


Figure 5.1 Plasma concentrations of prednisolone, prednisone, dihydroprednisolone and dihydroprednisone (mean $\pm$ se) in 12 horses following 5 days of oral dosing with prednisolone (1 g daily for 5 d)

The pharmacokinetics (PK) of prednisolone and its three metabolites are shown in Tables 5.2 to 5.5. Each compound fitted a 1-compartment PK model.

Following 5 days of oral dosing, plasma prednisolone concentrations peaked 40 min after administration according to the PK model (465 ng/mL; Table 5.2). Prednisolone concentrations then declined steadily to fall below the limit of detection within 24 h (Table 5.1). The half-life of prednisolone in plasma was 91 min ($t_{1/2\beta}$).

In comparison, the three metabolites peaked in plasma at a later time-point (between 68 and 171 min), but were also cleared rapidly and were all below the limit of detection within 24 h (Table 5.1). Plasma concentrations of the three metabolites were more variable between the horses when compared to the parent drug: half-lives ranged from 105 to 120 min.

Table 5.2 Pharmacokinetic parameters for prednisolone in plasma following the oral administration of prednisolone (1 g daily for 5 d) to 12 horses

	Mean $\pm$ SD	Min	Max	Median
Peak plasma concentration ($C_{\max}$), $\mu\text{g/L}$	465 $\pm$ 137	262	751	472
Time to peak plasma concentration ($T_{\max}$), h	0.67 $\pm$ 0.3	0.25	1.25	0.5
Apparent volume of distribution (V), L	1517 $\pm$ 474	865	2486	1377
Terminal half-life ($T_{1/2}$ for β), h	1.52 $\pm$ 0.53	0.8	2.3	1.53

Table 5.3 Pharmacokinetic parameters for prednisone in plasma following the oral administration of prednisolone (1 g daily for 5 d) to 12 horses

	Mean $\pm$ SD	Min	Max	Median
Peak plasma concentration ($C_{\max}$), $\mu\text{g/L}$	25.3 $\pm$ 6.67	13.2	37.0	24.5
Time to peak plasma concentration ($T_{\max}$), h	1.46 $\pm$ 0.76	0.75	3.5	1.25
Apparent volume of distribution (V), L	27576 $\pm$ 8443	17568	49242	26490
Terminal half-life ($T_{1/2}$ for β), h	2.00 $\pm$ 1.15	0.95	5.16	1.69

Table 5.4 Pharmacokinetic parameters for the metabolite dihydroprednisolone in plasma following the oral administration of prednisolone (1 g daily for 5 d) to 12 horses

	Mean $\pm$ SD	Min	Max	Median
Peak plasma concentration ($C_{\max}$), $\mu\text{g/L}$	948 $\pm$ 257	637	1370	870
Time to peak plasma concentration ($T_{\max}$), h	1.13 $\pm$ 0.35	0.75	1.75	1.13
Apparent volume of distribution (V), L	732 $\pm$ 188	475	1020	747
Terminal half-life ($T_{1/2}$ for β), h	1.75 $\pm$ 0.73	0.72	2.58	2.10

Table 5.5 Pharmacokinetic parameters for the metabolite dihydroprednisone in plasma following the oral administration of prednisolone (1 g daily for 5 d) to 12 horses

	Mean $\pm$ SD	Min	Max	Median
Peak plasma concentration ($C_{\max}$), $\mu\text{g/L}$	73.2 $\pm$ 25.7	30.4	116	61.7
Time to peak plasma concentration ($T_{\max}$), h	2.85 $\pm$ 1.01	1.25	5.0	3.00
Apparent volume of distribution (V), L	10113 $\pm$ 4260	5623	21382	10549
Terminal half-life ($T_{1/2}$ for β), h	2.00 $\pm$ 0.73	1.0	3.23	2.00

Urine prednisolone

Urine concentrations of prednisolone, prednisone, dihydroprednisolone and dihydroprednisone during the treatment period are shown in Table 5.6 and Fig. 5.2, and following treatment in Table 5.6 and Fig. 5.2.

Urinary excretion of prednisolone and all metabolites during the treatment period was highest on the 4th day of treatment (Fig. 5.2). Following cessation of treatment the urinary excretion pattern of prednisolone, prednisone and dihydroprednisolone was similar, with peak concentrations at 4.67 h

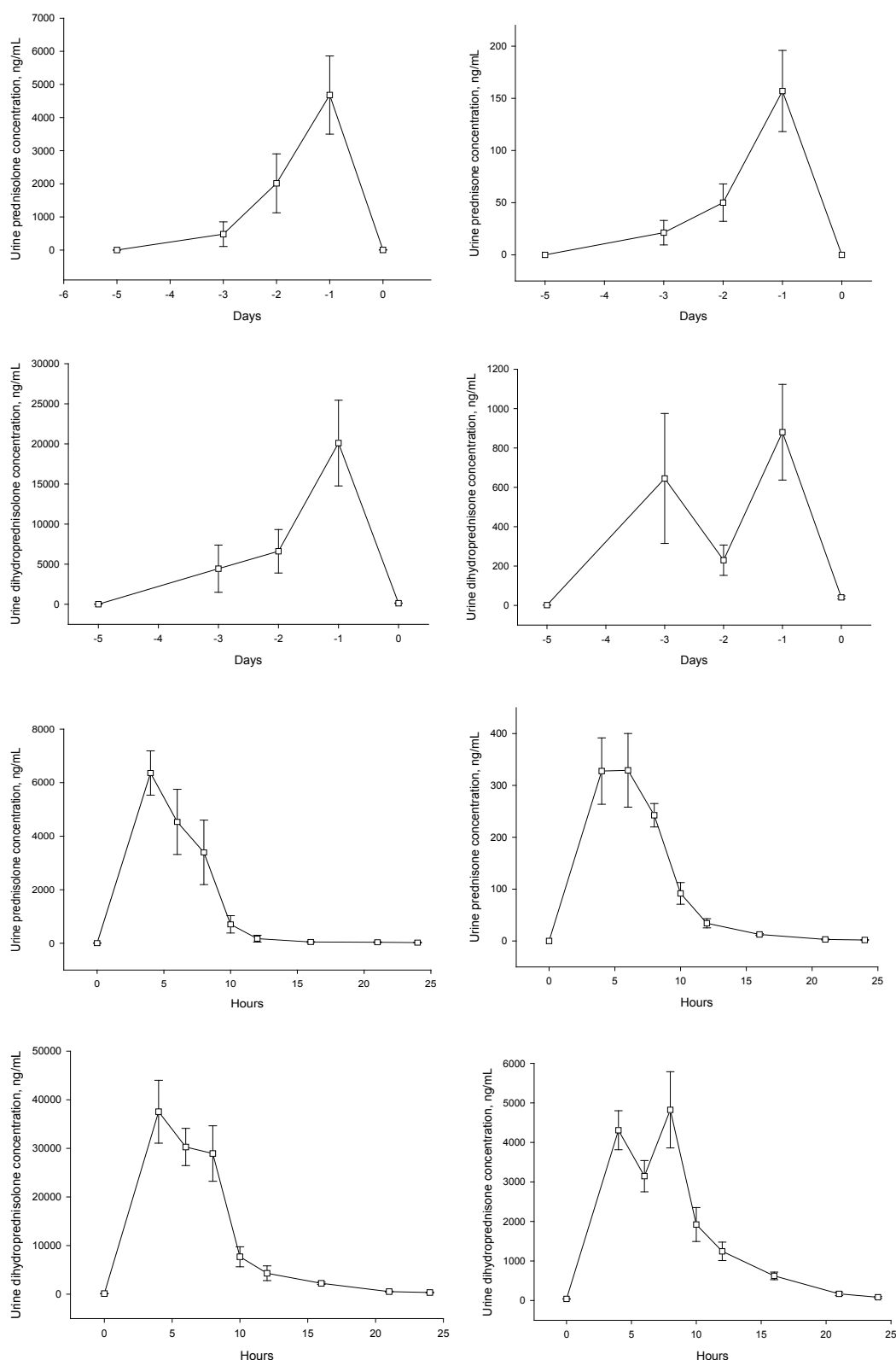


Figure 5.2 Urine concentrations of prednisolone, prednisone, dihydroprednisolone and dihydroprednisone (mean $\pm$ se) during and after 5 days of oral dosing (1 g daily for 5 d) in 12 horses, commencing on day -4

(Fig. 5.2). Dihydroprednisone excretion was slightly prolonged compared to the other 3 compounds and reached peak urine concentration at 8 h (Fig 5.2).

Urine concentrations of prednisolone, prednisone, and dihydroprednisone were below the limit of detection in most horses 48 h after the last dose of prednisolone, but were not undetectable in all horses until 72h. Dihydroprednisolone proved to be the most sensitive marker and remained detectable in urine up to 96 h in one horse (Table 5.6).

Table 5.6 Urine concentrations of prednisolone (mean $\pm$ se) and four metabolites following oral prednisolone (1 g daily for 5 d) administration to 12 horses

Time	Prednisolone (ng/mL)	Prednisone (ng/mL)	Dihydroprednisolone (ng/mL)	Dihydroprednisone (ng/mL)
-5 days	1.79 $\pm$ 1.05	0	12.3 $\pm$ 3.88	1.38 $\pm$ 1.27
-3 days	481 $\pm$ 371	21.3 $\pm$ 11.7	4442 $\pm$ 2946	645 $\pm$ 330
-2 days	2016 $\pm$ 890	50.0 $\pm$ 17.9	6611 $\pm$ 2712	230 $\pm$ 77.2
-1 days	4680 $\pm$ 1180	157 $\pm$ 38.9	20105 $\pm$ 5342	880 $\pm$ 243
0 h	4.74 $\pm$ 1.11	0	129 $\pm$ 22.8	40.7 $\pm$ 7.28
4 h	6360 $\pm$ 825	327.7 $\pm$ 64.0	37536 $\pm$ 6468	4308 $\pm$ 494
6 h	4535 $\pm$ 1217	329 $\pm$ 71.0	30281 $\pm$ 3828	3146 $\pm$ 395
8 h	3396 $\pm$ 1205	243 $\pm$ 22.6	28936 $\pm$ 5709	4824 $\pm$ 964
10 h	707 $\pm$ 323	91.8 $\pm$ 20.9	7700 $\pm$ 2060	1923 $\pm$ 430
12 h	172 $\pm$ 127	34.1 $\pm$ 8.82	4311 $\pm$ 1510	1244 $\pm$ 233
16 h	46.4 $\pm$ 16.5	12.6 $\pm$ 1.70	2240 $\pm$ 238	623 $\pm$ 96.2
21 h	38.6 $\pm$ 18.7	3.01 $\pm$ 0.95	534 $\pm$ 116	168 $\pm$ 44.2
24 h	25.9 $\pm$ 16.4	1.81 $\pm$ 0.91	346 $\pm$ 106	81.7 $\pm$ 21.6
28 h	2.37 $\pm$ 0.95	0	70.5 $\pm$ 14.1	25.5 $\pm$ 6.84
32 h	3.73 $\pm$ 2.72	1.38 $\pm$ 0.9	74.6 $\pm$ 23.6	21.7 $\pm$ 4.94
36 h	1.05 $\pm$ 0.47	0	19.6 $\pm$ 3.29	7.17 $\pm$ 2.64
40 h	3.29 $\pm$ 3.09	0.58 $\pm$ 0.58	15.7 $\pm$ 4.59	6.79 $\pm$ 5.47
48 h	0.92 $\pm$ 0.5	0	9.26 $\pm$ 3.64	0
72 h	0	0	2.8 $\pm$ 2.51	0
96 h	0	0	3.47 $\pm$ 2.97	0
120 h	0	-	-	-

Conclusion

Prednisolone is a potent anti-inflammatory and immunosuppressive steroid. Following 5 days of oral dosing, prednisolone concentrations in plasma peak within 40 min of ingestion, then decline steadily to fall below the limit of detection within 24 h. The prednisolone metabolite dihydroprednisolone may be detected in urine for up to 96 h. These data are currently being reviewed by the racing authorities with a view to determining appropriate detection times for oral prednisolone.

6 Methylprednisolone

Background

Methylprednisolone acetate (MPA) is a potent, long-acting corticosteroid that is used principally in veterinary medicine for its anti-inflammatory and immunosuppressive activity. It has glucocorticoid and mineralcorticoid action although it is reported to have less mineralcorticoid activity than both prednisolone and hydrocortisone.

Methylprednisolone acetate is administered to horses as a single intramuscular injection (200 mg) and sustains a prolonged duration of action over 2 to 3 weeks. In horses, MPA (Depo Medrol, Pfizer Animal Health and Ilium Depredil, Troy Laboratories) can be selected as a treatment for musculoskeletal pain and inflammation in both acute (tendonitis) and chronic (osteoarthritis) conditions (Goodrich and Nixon, 2006). The drug is also used to treat allergic conditions such as dermatitis. Methylprednisolone acetate can also be administered intra-synovially (120 mg) for joint disease.

Corticosteroids reduce inflammation by numerous mechanisms including inhibition of prostaglandin production and phospholipid release, and reducing the actions of neutrophils and eosinophils. They cannot be used concurrently with non-steroidal anti-inflammatory drugs, or in the presence of equine Cushing's disease (Schott, 2002), gastric ulceration, renal insufficiency or uncontrolled infections.

In horses, methylprednisolone reaches peak plasma concentrations 8 h after intra-articular injection of MPA and remains detectable in the plasma for as long as 196 h (Soma et al., 2006). However, while the characteristics of methylprednisolone have been described in plasma, urine and synovial fluid following intra-articular MPA administration in horses, there appears to be minimal pharmacokinetic data following IM routes of administration (Lillich et al., 1996; Soma et al., 2006). The aim of the current study was to model the pharmacokinetics of MPA following IM injection in 12 horses.

Methylprednisolone administration

Methylprednisolone acetate (Depo-Medrol, Pfizer Animal Health) was administered as a single IM injection to 12 Standardbred geldings (mean body weight 467 ± 9.76 kg) housed at The University of Queensland, Gatton Campus, Qld. The drug was given at a dose of 200 mg per horse (equivalent to 0.39 to 0.47 mg/kg).

Blood and urine samples were collected once prior to the MPA injection, then at regular intervals of decreasing frequency up to 60 days following dosing (Table 6.1).

Immediately after collection, blood and urine samples were centrifuged ($2,800 \times g$, 20 min). The plasma was divided into 2 portions and stored in plastic vials at -20°C . Urine samples were also divided into two portions and stored at -20°C . All samples were transported frozen to the Racing Science Centre, Qld, where they were analysed for the presence of methylprednisolone.

Extraction and analysis procedures

All samples were spiked with an internal standard prior to extraction. Urine samples were also subjected to enzyme hydrolysis using β -glucuronidase (*E. coli* type IX-A). Plasma and hydrolysed urine samples were extracted using an automated extraction method employing solid-phase extraction cartridges (UCT CEC18 500mg/3mL). Sample extracts are washed with a dilute caustic solution then

evaporated to dryness under dry nitrogen. The extracts were then reconstituted in acetonitrile/water (50:50 v/v) and analysed using LC-MS-MS.

Pharmacokinetic modelling

The pharmacokinetics of methylprednisolone in plasma were modelled at Charles Sturt University, NSW and The University of Pennsylvania, PA, using STATA Statistical Software 11.1 (Stata, 2007) as described in the general methods section. PK data are presented as the mean $\pm$ SD. All other data are presented as mean $\pm$ se.

Results and discussion

Plasma methylprednisolone

Concentrations of methylprednisolone are shown in Fig. 6.1 and Table 6.1. Plasma methylprednisolone concentrations peaked at 2.31 ng/mL 12 h 36 min after IM drug administration (Fig. 6.1). The pharmacokinetics of methylprednisolone in plasma fitted a single-compartment model, with a half-life ($t_{1/2\beta}$) of close to 303 h. These results are consistent with a long-acting lipophilic substance given by IM injection.

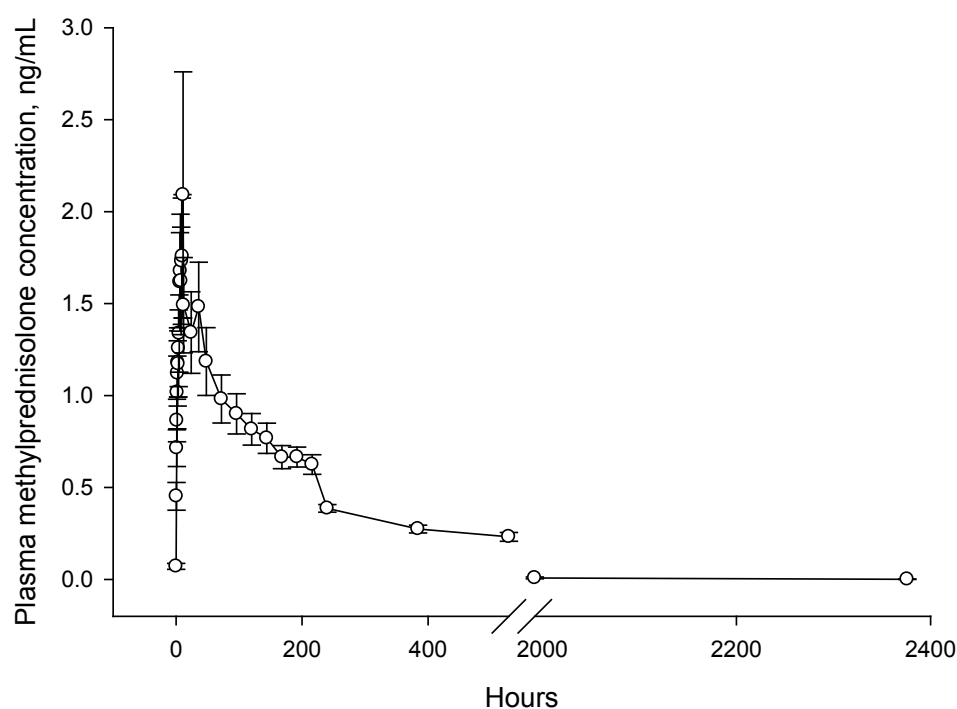


Figure 6.1 Plasma methylprednisolone concentrations (mean $\pm$ se) following intramuscular dosing with Depo-Medrol (200 mg) in 12 horses

Table 6.1 Plasma and urine concentrations (mean $\pm$ se) of methylprednisolone following intramuscular dosing with Depo-Medrol (200 mg) in 12 horses

Time (hours)	Plasma methylprednisolone concentration (ng/mL)	Urine methylprednisolone concentration (μ g/mL)
0	0.07 $\pm$ 0.02	0
0.5	0.45 $\pm$ 0.08	-
1	0.71 $\pm$ 0.10	-
1.5	0.86 $\pm$ 0.12	-
2	1.02 $\pm$ 0.20	16.6 $\pm$ 3.29
2.5	1.12 $\pm$ 0.18	-
3	1.18 $\pm$ 0.19	-
3.5	1.17 $\pm$ 0.18	-
4	1.26 $\pm$ 0.21	24.0 $\pm$ 6.22
5	1.34 $\pm$ 0.21	-
6	1.62 $\pm$ 0.27	33.1 $\pm$ 6.11
7	1.68 $\pm$ 0.31	-
8	1.62 $\pm$ 0.29	36.5 $\pm$ 8.11
9	1.73 $\pm$ 0.34	-
10	1.76 $\pm$ 0.34	-
11	2.09 $\pm$ 0.67	-
12	1.49 $\pm$ 0.26	50.0 $\pm$ 11.6
16	-	44.0 $\pm$ 7.83
24	1.34 $\pm$ 0.22	41.5 $\pm$ 7.05
36	1.48 $\pm$ 0.24	28.9 $\pm$ 7.53
48	1.19 $\pm$ 0.18	33.4 $\pm$ 6.71
72	0.98 $\pm$ 0.13	27.0 $\pm$ 5.48
96	0.90 $\pm$ 0.11	23.5 $\pm$ 4.77
120	0.82 $\pm$ 0.09	20.5 $\pm$ 4.93
144	0.77 $\pm$ 0.08	21.5 $\pm$ 4.52
168	0.67 $\pm$ 0.06	18.6 $\pm$ 3.49
192	0.67 $\pm$ 0.05	14.1 $\pm$ 3.45
216	0.63 $\pm$ 0.05	11.2 $\pm$ 3.03
240	0.39 $\pm$ 0.02	-
362	-	12.1 $\pm$ 2.40
384	0.27 $\pm$ 0.02	-
506	-	6.93 $\pm$ 1.39
528	0.23 $\pm$ 0.02	-
648	-	3.92 $\pm$ 0.88
696	0.15 $\pm$ 0.02	-
796	-	3.77 $\pm$ 1.08
840	0.10 $\pm$ 0.02	-
940	-	3.20 $\pm$ 0.98
1082	-	1.55 $\pm$ 0.64
1225	-	0.58 $\pm$ 0.24

1368	0.04 ± 0.01	-
1370	-	0.28 ± 0.12
1518	-	0.14 ± 0.06
1704	0.02 ± 0.01	-
1992	0.01 ± 0.005	-
2376	0.001 ± 0.001	-

Table 6.2 Pharmacokinetic parameters of methylprednisolone following intramuscular dosing with Depo-Medrol (200 mg) in 12 horses

	Mean $\pm$ SD	Min	Max	Median
Peak plasma concentration ($C_{\max}$), $\mu\text{g/L}$	2.31	0.58	8.96	1.52
Time to peak plasma concentration ($T_{\max}$), h	12.6 ± 8.72	6.00	36.00	10.00
Apparent volume of distribution (V), L	371 ± 238	55.3	853	327
Terminal half-life ($T_{1/2}$ for β), h	303 ± 243	107	981	205

Urine methylprednisolone

Concentrations of methylprednisolone in urine were much higher than those in plasma (Table 6.1 and Fig. 6.2), reaching a peak of between 17 and 171 $\mu\text{g/mL}$ in individual horses (mean $\pm$ se 76.3 ± 12 $\mu\text{g/mL}$) after an average of 25 h.

Following the peak, the concentrations fell relatively quickly over the first 8 d, then more slowly over the following period. Methylprednisolone was detectable in the urine of all horses for almost 5 weeks after administration, and was still detectable in some horses 9 weeks after administration (Fig. 6.2).

This prolonged excretion time was likely due to the very slow movement of methylprednisolone from the site of injection in muscle to the vascular space, because of the low solubility of methylprednisolone acetate. Less than 1% of the drug was recovered from urine during the first 48 h.

The urine: plasma ratio for methylprednisolone was estimated for the time points between 8 h and 216 h. The ratios for individual horses ranged from 21 to 35, with a mean ($\pm$ se) value of 28 ± 1.2 .

Further modelling and analysis of the urine data will allow the estimation of an appropriate detection time for forensic purposes

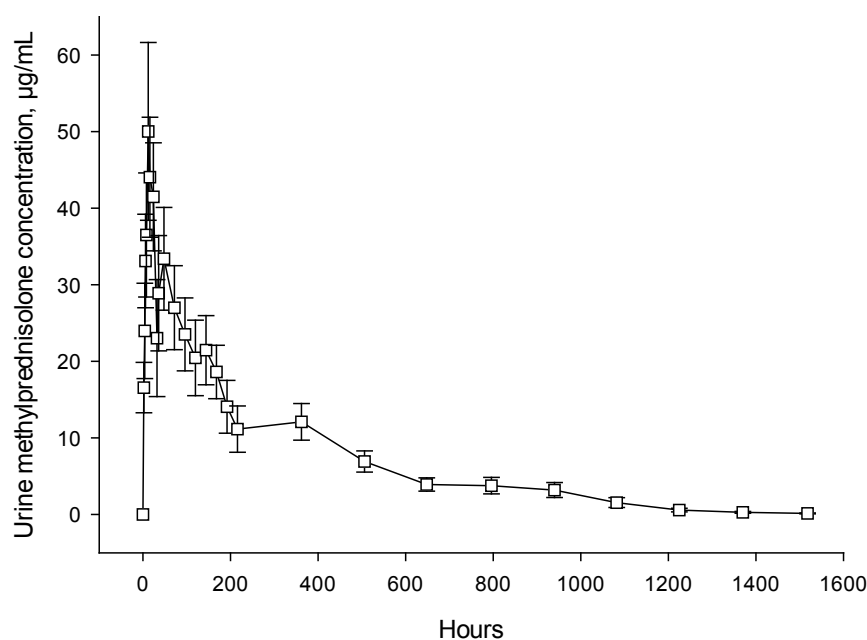


Figure 6.2 Urine methylprednisolone concentrations (mean $\pm$ se) following intramuscular dosing with Depo-Medrol (200 mg) in 12 horses

Conclusions

Methylprednisolone acetate is a long-acting corticosteroid used for its anti-inflammatory and immunosuppressive properties. When given in a slow-release formulation by IM injection its effects can be sustained for 2 to 3 weeks. Although plasma concentrations of methylprednisolone fall to low concentrations within 3 days, the drug can be detected in urine for up to 9 weeks. These data are currently being reviewed by the racing authorities with a view to determining an appropriate detection time for methylprednisolone.

7 Buscopan Compositum

Background

The active ingredients of the veterinary preparation Buscopan Compositum are hyoscine-N-butyl bromide (HBB, 4 mg/mL) and dipyrone (500 mg/mL). The therapeutic actions of HBB are principally anticholinergic and spasmolytic. The HBB works by decreasing the excitability of smooth muscle fibres and hence tone and peristaltic activity of the smooth muscles in hollow organs with parasympathetic innervation. This spasmolytic action is based on competitive inhibition of muscarinic receptors and ganglionic blockade of neuronal transmission.

Thus, HBB is used to treat spasmodic and impactive colic and oesophageal obstruction (choke) in horses (Gomaa et al., 2011). Buscopan is also beneficial at reducing the fluid loss associated with enteritis and diarrhoea via its spasmolytic action. It is very effective at relieving the pain associated with spasm and distension of the gastrointestinal, biliary and urogenital tracts (Roelvink et al., 1991). Dipyrone is a NSAID and thus assists with analgesia through the inhibition of prostaglandin synthesis, although it appears to have a less reliable analgesic effect than HBB (Roelvink et al., 1991).

Buscopan is available in Australia for slow IV injection in horses at a dose of 20 to 30 mL in horses and 5 to 15 mL in foals. It is marketed as Buscopan Compositum, Boehringer Ingelheim and Ilium Spasmogesic, Troy Laboratories (eMIMS IVS Australia, 2011).

Buscopan is poorly absorbed from the gastrointestinal system, but is rapidly distributed following IV injection and has a rapid onset of action (Gomaa et al., 2011). Upon IV administration, dipyrone is rapidly hydrolysed in plasma to 4-methylaminoantipyrine (MAA). The drug remains mainly in the enterohepatic circulation. Due to low lipid solubility Buscopan does not cross the blood brain barrier easily and is rarely associated with central nervous system side effects. Buscopan should not be administered concurrently with phenylbutazone.

Hyoscine-N-butyl bromide is excreted largely unchanged in urine, whereas dipyrone is metabolised to MAA, aminoantipyrine and formylaminoantipyrine (FAA). There is only one report of the excretion rate of HBB in horses (Popot et al., 2008) and in 2008 there were repeated incidences of racehorses returning positive urine test results to HBB following racing. The aims of the current study were to determine the pharmacokinetics, plasma concentrations and urine excretion times of HBB and MAA following IV administration of Buscopan Compositum.

Buscopan administration

Buscopan Compositum (Boehringer Ingelheim) was administered as a single IV injection to 12 geldings (5 Thoroughbred, 7 Standardbred, mean body weight 520 ± 11.4 kg) housed at Charles Sturt University, NSW. The drug was administered at a dose of 30 mL/horse, which delivered 120 mg HBB (equivalent to 0.21 to 0.27 mg/kg) and 15 g dipyrone (equivalent to 26 to 33 mg/kg).

Blood and urine samples were collected once prior to the Buscopan injection, then at regular intervals of decreasing frequency up to 48 h after dosing (Tables 7.1 and 7.4).

Immediately after collection, blood (20 mL) and urine (40 mL) samples were centrifuged (2,800 x g, 20 min). The plasma was divided into 2 portions and stored in plastic vials at -20°C . Urine samples were also divided into two portions and stored at -20°C . All samples were transported frozen to the Racing Science Centre, Qld, where they were analysed for the presence of HBB and dipyrone metabolites.

Extraction and analysis procedures

Urine and plasma samples for HBB analysis were extracted using an automated method employing solid-phase extraction cartridges (Waters Oasis WCX 60mg/3mL). Urine and plasma samples for MAA analysis underwent liquid/liquid extraction at pH 13-14 with di-isopropyl ether. All sample extracts were evaporated to dryness at 40 °C under nitrogen and then reconstituted in acetonitrile/water (50:50 v/v) for analysis by LC-MS-MS.

Pharmacokinetic modelling

The pharmacokinetics of HBB and MAA in plasma were modelled at Charles Sturt University, NSW and The University of Pennsylvania, PA, using WinSAAM software (<http://www.winsaam.com>, University of Pennsylvania) and STATA Statistical Software 11.1 (Stata, 2007) as described in the general methods section. PK data are presented as the mean $\pm$ SD. All other data are presented as mean $\pm$ se.

Results and discussion

Plasma Buscopan

The plasma concentrations and PK modelling of HBB are shown in Tables 7.1 and 7.2, respectively. Data for plasma MAA are shown in Tables 7.1 and 7.3.

Table 7.1 Plasma hyoscine butyl bromide (HBB) and plasma 4-methylaminoantipyrine (MAA) concentration (mean $\pm$ se) following intravenous dosing with Buscopan (30 mL) in 12 horses

Time	Plasma HBB (ng/mL)	Plasma MAA (ng/mL)
0 h	0	0
5 min	10995 $\pm$ 4996*	52392 $\pm$ 11600*
10 min	1985 $\pm$ 245	26958 $\pm$ 1387
20 min	913 $\pm$ 114	18267 $\pm$ 641
40 min	298 $\pm$ 29.5	13325 $\pm$ 765
1 h	139 $\pm$ 18.4	10811 $\pm$ 326
1.5 h	52.9 $\pm$ 9.82	9523 $\pm$ 369
2 h	20.2 $\pm$ 4.80	9393 $\pm$ 631
2.5 h	10.1 $\pm$ 3.22	7623 $\pm$ 425
3 h	9.04 $\pm$ 4.61	6784 $\pm$ 354
4 h	1.91 $\pm$ 1.53	5836 $\pm$ 218
6 h	0.28 $\pm$ 0.19	3913 $\pm$ 288
8 h	0	2676 $\pm$ 191
12 h	0	1296 $\pm$ 90.9
24 h	0	133 $\pm$ 40.3
32 h	0	9.25 $\pm$ 9.26
48 h	0	0

*Mean includes one apparent outlier observation

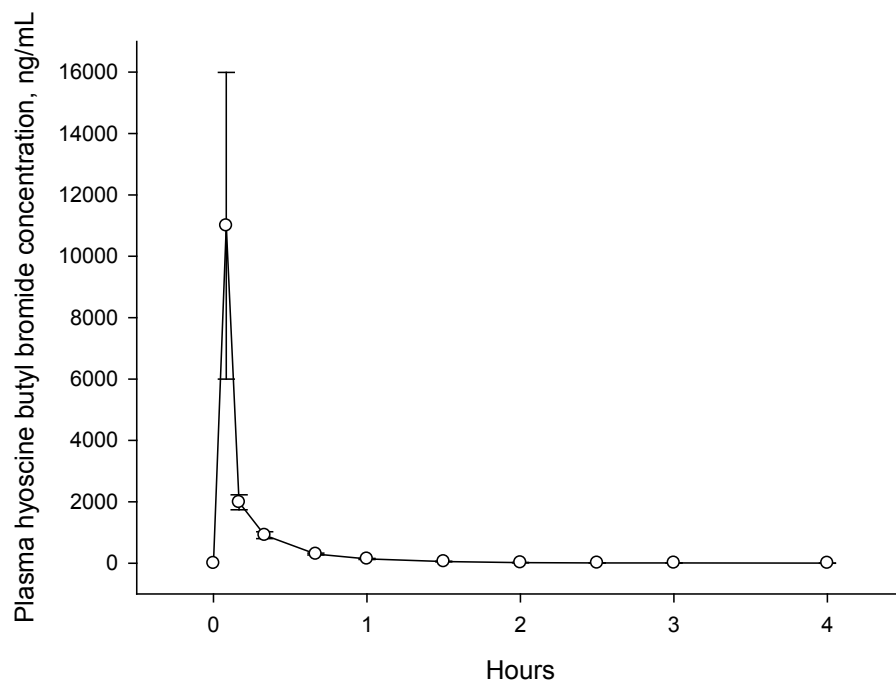


Figure 7.1 Plasma hyoscinebutylbromide concentrations (mean $\pm$ se) following intravenous dosing with Buscopan (30 mL) in 12 horses

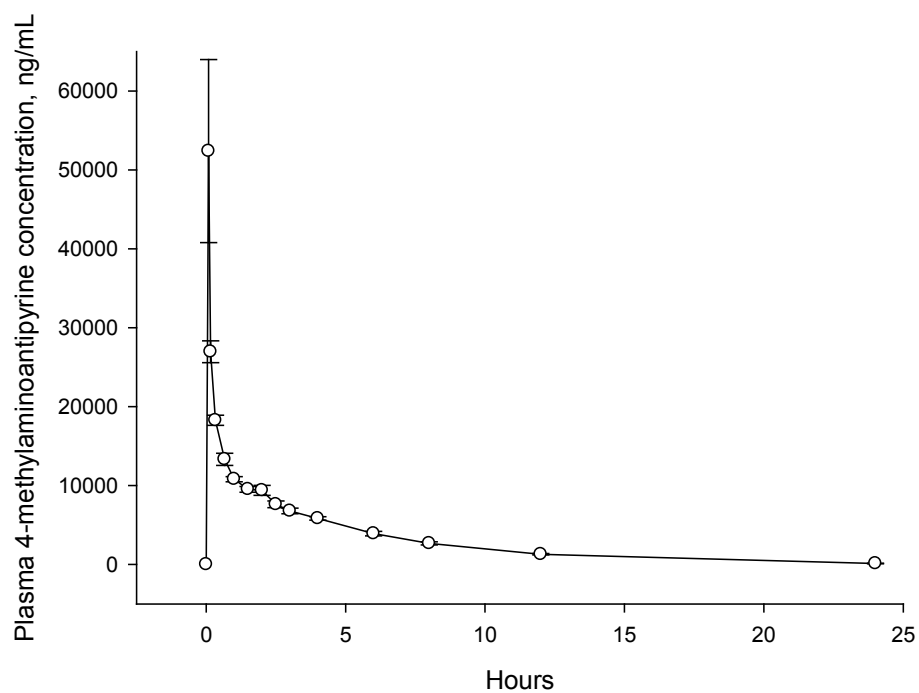


Figure 7.2 Plasma 4-methylaminoantipyrine concentrations (mean $\pm$ se) following intravenous dosing with Buscopan (30 mL) in 12 horses

Plasma HBB appeared to peak at 10995 ± 4996 ng/mL 5 min after administration (Table 7.1). However, closer examination of the data revealed that one horse had an unusually high concentration of Buscopan (65,400 ng/mL) resulting in an inflated mean and standard error (Fig 7.1). When this observation was excluded the mean $C_{\max}$ was only 6090 ng/mL (Table 7.2).

The drug fitted a 2-compartment model (Table 7.2) with plasma half lives of 0.08 ± 0.07 h for compartment one ($t_{1/2\alpha}$) and 0.45 ± 0.27 h for compartment two ($t_{1/2\beta}$). HBB levels in plasma were below the limit of detection in 3 horses 1.5 h after administration, but were not undetectable in all horses until 8 h after administration (Table 7.1).

Plasma concentrations of MAA were much higher than those of HBB with a peak of 52392 ± 11600 ng/mL also reached 5 min after Buscopan administration (Table 7.1). This set of observations also contained one unusually high value (from the same horse; 175,000 ng/mL) and when this observation was eliminated the mean became 41246 ± 10960 ng/mL.

The metabolite was largely cleared from the plasma 12 h after drug administration (Fig. 7.2), but was not below the limit of detection in all horses until 48 h (Table 7.1). MAA fitted a 2-compartment PK model (Table 7.3) and the half lives were 8.4 ± 3 min for compartment 1 ($t_{1/2\alpha}$) and 3.72 ± 0.05 h for compartment 2 ($t_{1/2\beta}$).

Table 7.2 Pharmacokinetic parameters for hyoscine butyl bromide (HBB) following the administration of Buscopan (30 mL) to 11* horses

	Mean $\pm$ SD	Min	Max	Median
Peak plasma concentration ($C_{\max}$), μ g/L	6090 ± 667	2380	9970	6445
Time to peak plasma concentration ($T_{\max}$), min	5 ± 0	5	5	5
Apparent volume of distribution (VT), L/kg	0.05 ± 0.03	0.02	0.12	0.04
Compartment 1				
Terminal half-life ($T_{1/2}$ for α), h	0.08 ± 0.07	0.03	0.24	0.05
Compartment 2				
Terminal half-life ($T_{1/2}$ for β), h	0.45 ± 0.27	0.15	0.37	0.37

*Data from one horse were not able to be modelled

Table 7.3 Pharmacokinetic parameters for 4-methylaminoantipyrine (MAA) after administration of Buscopan (30 mL) to 12 horses

	Mean $\pm$ SD	Min	Max	Median
Peak plasma concentration ($C_{\max}$), μ g/L	41246 ± 10960	27200	61400	43023
Time to peak plasma concentration ($T_{\max}$), min	5 ± 0	5	5	5
Apparent volume of distribution (V), L	100 ± 14.4	74	118	106
Apparent volume of distribution (VT), L/kg	0.19 ± 0.03	0.14	0.23	0.20
Compartment 1				
Terminal half-life ($T_{1/2}$ for α), h	0.14 ± 0.05	0.07	0.26	0.14
Compartment 2				
Terminal half-life ($T_{1/2}$ for β), h	3.72 ± 0.05	3.02	4.46	3.63

Urine Buscopan

Urine concentrations of three dipyrone metabolites: MAA, AA and FAA, are shown in Table 7.4 and Figure 7.3.

Peak urinary excretion of the dipyrone metabolites occurred 2 to 4 h after Buscopan administration (Fig. 7.3a). The urine concentrations declined to low levels after 48 h, but aminoantipyrine (AA) and formylaminoantipyrine (FAA) were not below the limit of detection in all horses until 72 h.

The metabolite 4-methylaminoantipyrine (MAA) was detected in 9 of the 12 horses at 72 h and was present in one horse for up to 240 h. The low mean concentration of MAA beyond 48 h, as shown in Table 7.4, reflects the fact that MAA was undetectable in most horses beyond this time. No metabolites were detected in any horse after 312 h.

Table 7.4 Urine concentrations of 4-methylaminoantipyrine (MAA), aminoantipyrine (AA) and formylaminoantipyrine (FAA) (mean $\pm$ se) following intravenous dosing with Buscopan (30 mL) in 12 horses

Time (hours)	Urine MAA ($\mu\text{g/mL}$)	Urine AA ($\mu\text{g/mL}$)	Urine FAA ($\mu\text{g/mL}$)
0	0	0	0
2	589 $\pm$ 54.5	47.0 $\pm$ 6.61	15.0 $\pm$ 2.15
4	502 $\pm$ 51.6	133 $\pm$ 17.5	55.5 $\pm$ 10.4
6	318 $\pm$ 32.2	78.4 $\pm$ 6.46	33.8 $\pm$ 4.81
8	172 $\pm$ 19.2	41.7 $\pm$ 6.29	26.2 $\pm$ 4.47
12	95.4 $\pm$ 10.7	41.1 $\pm$ 6.29	35.1 $\pm$ 6.28
24	38.6 $\pm$ 7.64	14.1 $\pm$ 2.90	15.6 $\pm$ 3.51
32	5.48 $\pm$ 1.19	2.07 $\pm$ 0.40	3.78 $\pm$ 1.15
36	-	-	-
40	-	-	-
44	-	-	-
48	1.32 $\pm$ 0.59	0.60 $\pm$ 0.19	0.32 $\pm$ 0.32
72	0.09 $\pm$ 0.02	-	-
96	0.03 $\pm$ 0.02	-	-
120	0.02 $\pm$ 0.01	-	-
144	0.02 $\pm$ 0.02	-	-
168	0.02 $\pm$ 0.01	-	-
192	0.01 $\pm$ 0.01	-	-
216	0.01 $\pm$ 0.01	-	-
240	0.01 $\pm$ 0.01	-	-

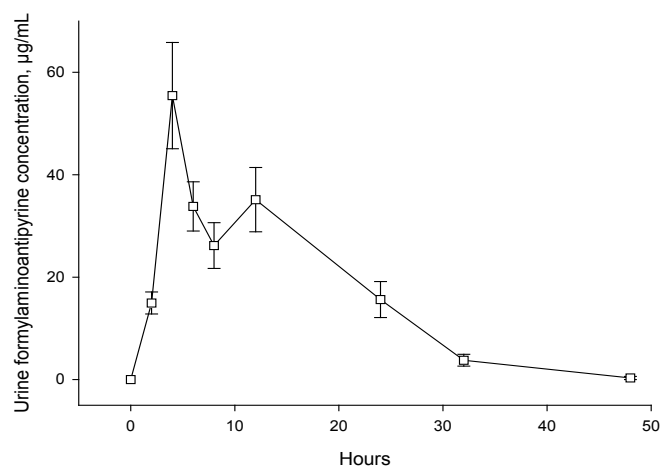
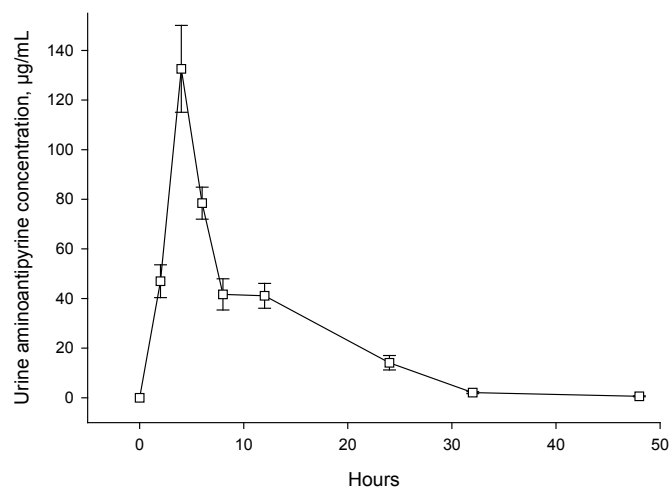
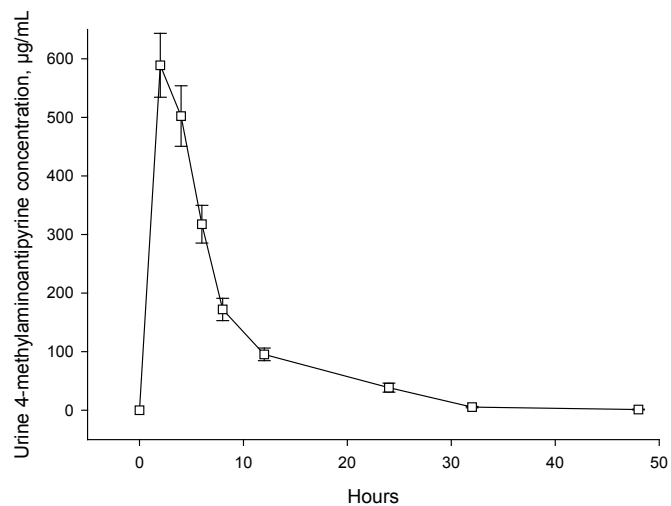


Figure 7.3 Urine concentrations of three dipyrone metabolites (mean $\pm$ se) following intravenous dosing with Buscopan (30 mL) in 12 horses

Conclusions

Buscopan is a composite drug containing two active ingredients (HBB and dipyrone) with spasmolytic and anti-inflammatory actions.

When given by IV injection, peak concentrations of HBB are reached within 5 min and the drug is largely cleared from plasma within 90 min. Two dipyrone metabolites (AA and FAA) fall below the limit of detection in urine 72 h after Buscopan administration, whereas a third metabolite (MAA) may be detected for up to 240 h.

These data are currently being reviewed by the racing authorities with a view to developing appropriate reporting levels for Buscopan.

8 Lignocaine

Background

Lignocaine is an amide type of local anaesthetic commonly used in equine veterinary medicine to facilitate the diagnosis of lameness following nerve blockade, regional anaesthesia to allow minor surgical procedures or dentistry, epidural anaesthesia and for pain relief (Doherty and Seddighi, 2010; Hubbell et al., 2010). The drug stabilises excitable membranes and induces a reversible blockade of local nerve conduction which results in analgesia, relaxation and hyporeflexia of the treated area. Constant rate infusions of lignocaine have also been reported to be safe for prolonged local anaesthesia in horses (Dickey et al., 2008).

Lignocaine is normally formulated at a concentration of 2% and can be formulated with or without adrenaline. It is available in Australia: Ilium Lignocaine 20 Injection (Troy Laboratories) and Lignomav 2% Injection (Mavlab). The principal route of administration is by SC or intradermal injection. Dose rates are 'to effect' but a maximum dose will usually be given for each product for each use in horses (e.g. 20 mL for intradermal or SC use). Overdoses can result in cardiac complications such as hypotension and reduced heart rate. Inadvertent IV administration may cause central nervous system disturbances such as excitement or convulsions (eMIMS IVS Australia, 2011).

Local anaesthetic drugs are metabolised by the liver and eliminated via renal excretion. Lignocaine is converted to several metabolites including monoethylglycinexylidide (MEGX), glycinexylidide (GX), 3-hydroxy-lignocaine (3-OH-lignocaine) and 4-hydroxy-lignocaine (4-OH-lignocaine). The PK of lignocaine have been well studied following IV infusion in horses (Dickey et al., 2008; Milligan et al., 2006). There is less data on the plasma and urine disposition of lignocaine following SC injection, however, it has been shown to be rapidly absorbed and eliminated in one study using Icelandic horses (Kristinsson et al., 1996). This was in agreement with another study which showed that one metabolite of lignocaine was also rapidly eliminated (Harkins et al., 1998). The aim of the current study was to examine the plasma and urine disposition of lignocaine following SC administration in 12 horses.

Lignocaine administration

Lignocaine (Ilium Lignocaine 20, Troy Laboratories) was administered as a single SC injection at a dose of 0.8 mg/kg to 12 Standardbred geldings (mean body weight 497 ± 12.2 kg) housed at The University of Queensland, Gatton Campus, Qld. Blood and urine samples were collected once prior to the lignocaine injection, then at regular intervals of decreasing frequency up to 120 h after dosing (Tables 8.1 and 8.2).

Immediately after collection, blood (40 mL) and urine (40 mL) samples were centrifuged ($2,800 \times g$, 10 min). The plasma was divided into 2 portions and stored in plastic vials at -20°C . Urine samples were also divided into two portions and stored at -20°C . All samples were transported frozen to the Racing Science Centre, Qld, where they were analysed for the presence of lignocaine and its metabolites (Nelis et al., 2010).

Extraction and analysis procedures

All samples were spiked with internal standards. Urine samples were also subjected to enzyme hydrolysis with β -glucuronidase/arylsulphatase prior to extraction. The pH of the urine and plasma samples was adjusted to 5.8 using HCl and/or ammonia and the samples were aliquoted into glass tubes. The extraction process involved the separation of both acidic and basic fractions and employed solid-phase extraction cartridges (UCT CSDAU503 500mg/3mL) containing mixed-mode packing

material and mounted in an automated workstation. Acidic and basic analytes were fractionated and collected into separate tubes. All sample eluents were evaporated to dryness at 40 °C under nitrogen and then reconstituted in acetonitrile/water (50:50 v/v) before analysis using LC-MS-MS.

Pharmacokinetic modelling

The pharmacokinetics of lignocaine were modelled at Charles Sturt University, NSW and The University of Pennsylvania, PA, using STATA Statistical Software 11.1 (Stata, 2007) as described in the general methods section. Modelling was constrained by not knowing the lignocaine bioavailability as the route of administration was not intravenous and no published bioavailability data are available for horses. PK Data are presented as the mean $\pm$ SD. All other data are presented as mean $\pm$ se.

Results and discussion

Plasma lignocaine

Plasma concentrations of lignocaine and its metabolite are shown in Figure 8.1, 8.2 and Table 8.1.

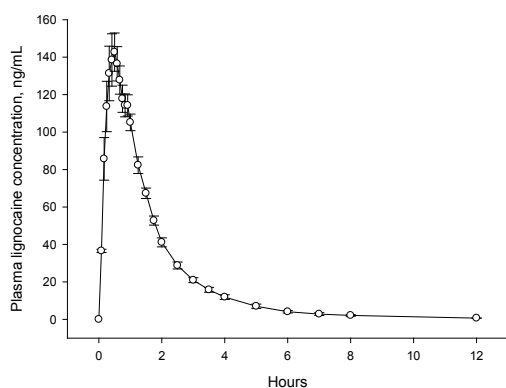


Figure 8.1 Plasma lignocaine concentrations (mean $\pm$ se) after subcutaneous dosing (0.8 mg/kg) in 12 horses

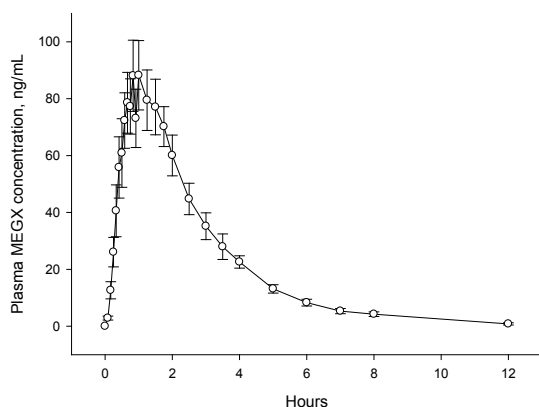


Figure 8.2 Plasma monoethylglycinexylidide (MEGX) concentrations (mean $\pm$ se) after subcutaneous dosing with lignocaine (0.8 mg/kg) in 12 horses

Table 8.1 Plasma concentrations of lignocaine and monoethylglycinexylidide (MEGX) (mean $\pm$ se) in 12 horses after subcutaneous dosing with lignocaine (0.8 mg/kg)

Time	Plasma lignocaine (ng/mL)	Plasma MEGX (ng/mL)
0 h	0	0
5 min	36.6 $\pm$ 0.85	2.86 $\pm$ 0.69
10 min	85.7 $\pm$ 11.4	12.6 $\pm$ 3.01
15 min	114 $\pm$ 13.4	26.1 $\pm$ 5.17
20 min	131 $\pm$ 14.6	40.6 $\pm$ 9.10
25 min	139 $\pm$ 14.0	55.8 $\pm$ 10.8
30 min	143 $\pm$ 10.2	60.9 $\pm$ 12.1
35 min	136 $\pm$ 9.18	72.3 $\pm$ 9.75
40 min	128 $\pm$ 7.52	78.6 $\pm$ 10.7
45 min	118 $\pm$ 7.23	77.3 $\pm$ 9.73
50 min	114 $\pm$ 6.10	88.1 $\pm$ 12.5
55 min	114 $\pm$ 5.64	73.1 $\pm$ 10.3
1 h	105 $\pm$ 4.42	88.2 $\pm$ 12.2
1.25 h	82.3 $\pm$ 4.44	79.5 $\pm$ 10.6
1.5 h	67.3 $\pm$ 2.79	77.1 $\pm$ 9.77
1.75 h	52.8 $\pm$ 2.42	70.2 $\pm$ 7.01
2 h	41.1 $\pm$ 2.38	60.1 $\pm$ 7.17
2.5 h	28.7 $\pm$ 1.83	44.8 $\pm$ 5.53
3 h	20.9 $\pm$ 1.32	35.2 $\pm$ 4.69
3.5 h	15.8 $\pm$ 1.30	28.0 $\pm$ 4.49
4 h	11.9 $\pm$ 1.23	22.6 $\pm$ 2.16
5 h	7.02 $\pm$ 1.15	13.1 $\pm$ 1.48
6 h	4.07 $\pm$ 0.68	8.31 $\pm$ 1.18
7 h	2.84 $\pm$ 0.78	5.33 $\pm$ 0.91
8 h	2.09 $\pm$ 0.45	4.23 $\pm$ 0.81
12 h	0.67 $\pm$ 0.11	0.83 $\pm$ 0.38
24 h	0.16 $\pm$ 0.04	0.11 $\pm$ 0.06
36 h	0.10 $\pm$ 0.03	0.05 $\pm$ 0.03
48 h	0	0.05 $\pm$ 0.03
72 h	0	0.06 $\pm$ 0.04
96 h	0	0
120 h	0	0

Following subcutaneous injection plasma concentrations of lignocaine peaked at an average of 155 ng/mL after an average 30 min (Fig. 8.1, Table 8.2). In contrast, the MEGX concentration did not peak (88.2 $\pm$ 12.2 ng/mL) until 1 h after lignocaine injection (Fig. 8.2).

Plasma lignocaine concentrations were below the limit of detection in 11 horses within 24 h and were undetectable in all horses by 36 h. The metabolite MEGX was below the limit of detection in 8 horses by 24 h, but was still detectable in one horse for up to 72 h after lignocaine administration (Table 8.1). There was more variation in plasma concentrations of MEGX than lignocaine between individual horses (Figs. 8.1 and 8.2).

Table 8.2 Pharmacokinetic parameters for lignocaine following the subcutaneous administration of lignocaine (0.8 mg/kg) to 12 horses

	Mean ± SD	Min	Max	Median
Peak plasma concentration (C _{max}), µg/L	155 ± 41.5	103	262	144
Time to peak plasma concentration (T _{max}), min	30.3 ± 8.4	16	46	30
Apparent volume of distribution (VT), L/kg	Not calculated – bioavailability not known			
Compartment 1				
Terminal half-life (T _{1/2} for α), min*	6 ± 2.4	1.44	9	6
Compartment 2				
Terminal half-life (T _{1/2} for β), min*	65 ± 15.6	48	110.4	61.8

*Assumes a bioavailability of approximately 25%

Lignocaine pharmacokinetics followed a two-compartment model, with half-lives of approximately 6 min and 56 min for the two compartments. These values assume a bioavailability of 25%, but are relatively independent of bioavailability/dose rate.

Urine lignocaine

The concentrations of lignocaine and its metabolites: MEGX, GX, 3-OH-lignocaine and 4-OH-lignocaine in urine are reported in Table 8.3 and Figure 8.3.

Table 8.3 Urine concentrations of lignocaine and its metabolites MEGX, GX, 3-HO lignocaine and 4-HO lignocaine (mean $\pm$ se) after subcutaneous dosing with lignocaine (0.8 mg/kg) in 12 horses

Time (h)	Urine lignocaine (ng/mL)	Urine MEGX (ng/mL)	Urine GX (ng/mL)	Urine 3-HO-Lignocaine (ng/mL)	Urine 4-HO-Lignocaine (ng/mL)
0	0	0	0	0	0
2	21.5 $\pm$ 5.20	758 $\pm$ 180	118 $\pm$ 47.3	2172 $\pm$ 374	258 $\pm$ 64.3
4	48.0 $\pm$ 7.58	1408 $\pm$ 346	184 $\pm$ 798.	2020 $\pm$ 178	254 $\pm$ 80.5
6	48.2 $\pm$ 26.5	633 $\pm$ 229	135 $\pm$ 42.0	-	194 $\pm$ 37.8
8	20.2 $\pm$ 5.09	1064 $\pm$ 355	111 $\pm$ 14.5	1793 $\pm$ 269	183 $\pm$ 16.6
12	21.6 $\pm$ 10.7	574 $\pm$ 147	71.4 $\pm$ 16.6	1335 $\pm$ 214	152 $\pm$ 26.0
16	2.48 $\pm$ 0.74	80.6 $\pm$ 21.5	9.28 $\pm$ 2.12	216 $\pm$ 37.8	22.2 $\pm$ 5.44
24	1.98 $\pm$ 1.02	47.2 $\pm$ 26.9	4.93 $\pm$ 2.31	65.4 $\pm$ 16.0	5.44 $\pm$ 1.67
36	0.19 $\pm$ 0.01	4.36 $\pm$ 1.63	0.24 $\pm$ 0.08	13.6 $\pm$ 2.54	0.91 $\pm$ 0.13
48	0.31 $\pm$ 0.11	1.10 $\pm$ 0.28	0.07 $\pm$ 0.06	3.89 $\pm$ 1.80	0.39 $\pm$ 0.13
72	0.08 $\pm$ 0.02	0.35 $\pm$ 0.09	0.01 $\pm$ 0.01	0.75 $\pm$ 0.17	0.07 $\pm$ 0.01
96	0.12 $\pm$ 0.05	0.22 $\pm$ 0.04	0.03 $\pm$ 0.03	0.48 $\pm$ 0.10	0.11 $\pm$ 0.04
120	0.08 $\pm$ 0.01	0.17 $\pm$ 0.04	0.03 $\pm$ 0.02	0.36 $\pm$ 0.15	0.09 $\pm$ 0.05

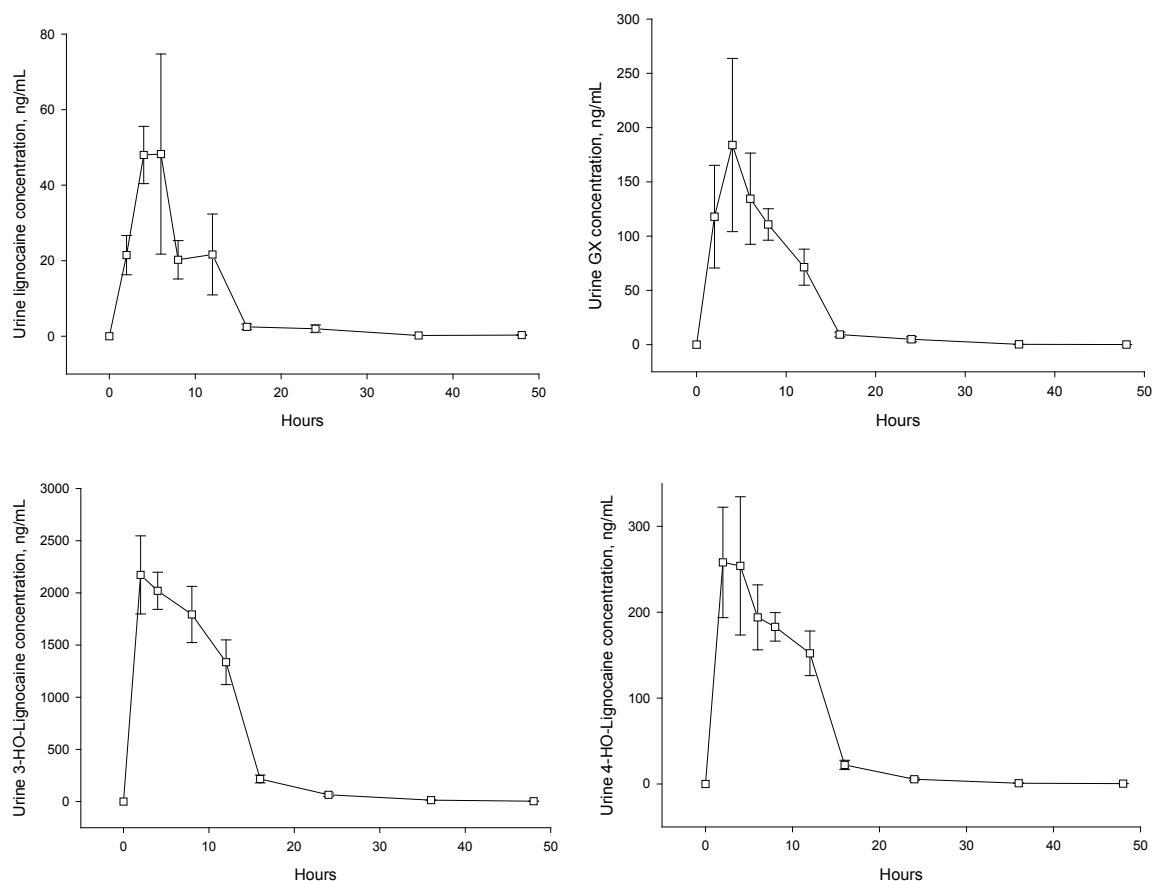


Figure 8.3 Urine concentrations of lignocaine and four metabolites (mean $\pm$ se) after subcutaneous dosing with lignocaine (0.8 mg/kg) in 12 horses

Lignocaine concentrations peaked in the urine 6 h after administration at 48.2 ng/mL (Fig. 8.3) and the drug was largely excreted by 48 h. However, lignocaine concentrations did not fall below the limit of detection in all horses until 120h.

The metabolites reached peak concentrations at similar times to the parent drug (Fig. 8.3) although at markedly higher concentrations (Table 8.2). The metabolites were detectable at low concentrations in at least one horse for up to 120 h after lignocaine administration.

Conclusions

Lignocaine is a commonly-used local anaesthetic agent often given by subcutaneous injection. When given by this route plasma concentrations peak within 30 min and most of the drug is cleared from plasma by 6 to 12 h. Metabolites of lignocaine can be detected in urine at low concentrations in some horses for up to 120 h, however. The racing authorities are currently reviewing these data with a view to setting appropriate reporting levels for lignocaine and its metabolites.

9 Mepivacaine

Background

Mepivacaine is an iso-osmotic amide local anaesthetic drug that is used in horses to facilitate the diagnosis of lameness (with nerve blockade) and during surgical procedures (Bidwell et al., 2004). It is reported to be associated with less tissue irritation than lignocaine and thus may be preferable for use in minor wound surgery, particularly in the lower limb where wound healing is often compromised. It is also used for epidural anaesthesia and spinal/intrathecal anaesthesia. Mepivacaine is marketed principally for use in horses and is commonly used in veterinary practice (Hubbell et al., 2010).

Mepivacaine is formulated as a 2% solution (20 mg/mL) for SC or IM injection. The volume administered is dependent on response, but is approximately 5 to 10 mL for perineural anaesthesia (nerve blocks) and 8 to 10 mL for low epidural. The drug is available in Australia as Mepivacaine injection (Nature Vet) and Ilium Vetcaine (Troy Laboratories) (eMIMS IVS Australia, 2011).

Mepivacaine has a more rapid onset of action than lignocaine and is reported to produce more prolonged anaesthesia than either lignocaine or prilocaine. It has been shown to have good local tissue distribution following intra-articular injection in horses (Keegan et al., 1996).

Local anaesthetic drugs are metabolised by the liver and eliminated via renal excretion. Mepivacaine is metabolised to 3-hydroxy-mepivacaine and 4-hydroxy-mepivacaine. The pharmacokinetics of mepivacaine following SC injection have not been studied widely in horses (Harkins et al., 1999). The aim of the current study was to examine the plasma and urine disposition of mepivacaine and its metabolites following SC injection in 12 horses.

Mepivacaine administration

Mepivacaine (Mepivacaine, Nature Vet) was administered as a single SC injection (400 mg; 0.68 to 0.99 mg/kg) to 12 Standardbred geldings (mean body weight 498 ± 16.7 kg) housed at The University of Queensland, Gatton Campus, Qld.

Blood and urine samples were collected once prior to the mepivacaine injection, then at regular intervals of decreasing frequency for up to 96 h after dosing (Table 9.1).

Immediately after collection, blood (40 mL) and urine (40 mL) samples were centrifuged (2,800 x g, 10 min). The plasma was divided into 2 portions and stored in plastic vials at -20°C . Urine samples were also divided into two portions and stored at -20°C . All samples were transported frozen to the Racing Science Centre, Qld, where they were analysed for the presence of mepivacaine and its metabolites.

Extraction and analysis procedures

All samples were spiked with internal standards. Urine samples were also subjected to enzyme hydrolysis with β -glucuronidase/arylsulphatase prior to extraction. The pH of the urine and plasma samples was adjusted to 5.8 using HCl and/or ammonia and the samples were aliquoted into glass tubes. The extraction process involved the separation of both acidic and basic fractions and employed solid-phase extraction cartridges (UCT CSDAU503 500mg/3mL) containing mixed-mode packing material and mounted in an automated workstation. Acidic and basic analytes were fractionated and

collected into separate tubes. All sample eluents were evaporated to dryness at 40°C under nitrogen and then reconstituted in acetonitrile/water (50:50 v/v) before analysis using LC-MS-MS.

Pharmacokinetic modelling

In contrast to the other therapeutic substances described in this report, an initial examination showed that the pharmacokinetics of Mepivacaine were non-linear, and so a different modelling approach was adopted.

The concentration-time data for mepivacaine in plasma were analysed by nonlinear mixed-effects modelling (NONMEM version 6.1, Globomax LLC, USA). A Digital Fortran compiler was used and the runs were executed using Wings for NONMEM. The model was fitted to the data using the first-order conditional estimation method with interaction and ADVAN6 to solve the differential equations. Between-subject variability (BSV) was calculated using an exponential variability model and was assumed to follow a lognormal distribution. Residual unexplained variability (RUV) was modelled using a combined exponential and additive random error. Visual inspection of diagnostic scatter plots and the NONMEM objective function value (OBJ) were used to assess goodness of fit. Statistical comparison of nested models was undertaken on the basis of a χ^2 test of the difference in OBJ. A decrease in the OBJ of 3.84 units ($P < 0.05$) was considered statistically significant. A forwards and backwards stepwise approach was used to include body weight into the model. This covariate was included if it was biologically plausible and if the decrease in OBJ was at least 3.84 units.

The model in plasma was evaluated by performing a visual predictive check (VPC), and by nonparametric bootstrapping with re-sampling and replacement. For the VPC, 1000 data sets were simulated from the final parameter estimates using the original data as a template. The 5th, 50th and 95th percentiles of simulated concentrations were then computed and plotted against observations. A nonparametric bootstrap method was used to assess the uncertainty in parameter estimates. The 2.5th, 50th and 97.5th percentiles were then calculated from the empirical posterior distribution of 1000 bootstrap replicates.

The modelling was conducted by The University of Queensland in collaboration with Monash University. PK data are presented as the mean $\pm$ SD. All other data are presented as mean $\pm$ se.

Results and discussion

Plasma mepivacaine

Plasma concentrations of mepivacaine are shown in Table 9.1 and Figure 9.1. Mepivacaine concentrations peaked in the plasma (113 ng/mL) 1 h after dosing, before declining steadily (Fig. 9.1). Mepivacaine was detectable in plasma in all horses 12 h after administration, but was not detectable in any horse 24 h after administration (Table 9.1).

The PK parameters for plasma mepivacaine calculated from models fitted to individual horses are shown in table 9.2. The parameters for the final model derived from bootstrapping 1000 replicates are shown in Table 9.3. After screening for the effect of body weight on clearance rate and volume of distribution, no statistically significant improvements in the model were found. Accordingly, body weight was not included as a factor in the final PK model.

The final estimate for the rate of mepivacaine absorption was 31 min. At a median concentration of 76 $\mu\text{g/L}$ for all 12 horses, the model of mixed-order elimination gave a clearance rate of 629 L/h. A 1-compartment model described the disposition of mepivacaine, with a large central volume of distribution (3358 L) consistent with a drug of high lipophilicity.

Table 9.1 Plasma and urine concentrations of mepivacaine, and concentrations of two mepivacaine metabolites in urine (mean $\pm$ se) after dosing with mepivacaine (400 mg, SC) in 12 horses

Time	Plasma mepivacaine (ng/mL)	Urine mepivacaine (ng/mL)	Urine 3-hydroxy mepivacaine (μ g/mL)	Urine 4-hydroxy mepivacaine (ng/mL)
0 h	0	0	0	0
15 min	36.8 $\pm$ 4.67	-	-	-
20 min	91.7 $\pm$ 13.1	-	-	-
25 min	101 $\pm$ 10.2	-	-	-
30 min	103 $\pm$ 8.88	-	-	-
35 min	104 $\pm$ 9.05	-	-	-
40 min	107 $\pm$ 7.79	-	-	-
50 min	109 $\pm$ 9.08	-	-	-
1 h	113 $\pm$ 5.97	-	-	-
70 min	112 $\pm$ 7.56	-	-	-
85 min	104 $\pm$ 8.27	-	-	-
100 min	104 $\pm$ 7.83	-	-	-
2 h	95.6 $\pm$ 8.77	-	-	-
2 h 10 min	89.3 $\pm$ 8.12	-	-	-
2 h 40 min	86.1 $\pm$ 7.56	-	-	-
3 h		77.1 $\pm$ 20.7	4.70 $\pm$ 2.39	542 $\pm$ 189
13 h 10 min	69.2 $\pm$ 5.86	-	-	-
3 h 40 min	63.3 $\pm$ 5.14	-	-	-
4 h 10 min	60.9 $\pm$ 4.17	-	-	-
5 h		144 $\pm$ 46.9	6.36 $\pm$ 1.46	1180 $\pm$ 383
5 h 10 min	49.8 $\pm$ 3.53	-	-	-
6 h 10 min	38.4 $\pm$ 2.86	-	-	-
7 h 10 min	28.6 $\pm$ 2.73	-	-	-
8 h		125 $\pm$ 38.5	34.6 $\pm$ 1.38	1686 $\pm$ 558
8 h 10 min	18.2 $\pm$ 2.46	-	-	-
10 h		115 $\pm$ 30.7	16.8 $\pm$ 8.37	1161 $\pm$ 371
12 h	3.94 $\pm$ 0.73	41.2 $\pm$ 10.4	9.15 $\pm$ 2.75	738 $\pm$ 178
16 h		24.0 $\pm$ 8.7	3.15 $\pm$ 0.62	309 $\pm$ 54.5
24 h	0	3.87 $\pm$ 1.16	0.69 $\pm$ 0.18	63.7 $\pm$ 16.7
36 h	0	-	-	-
48 h	0	0	0.07 $\pm$ 0.04	1.78 $\pm$ 1.38
72 h	0	0	0.01 $\pm$ 0.003	0
96 h	0	0	0	0

Note that concentrations of 3-hydroxy mepivacaine are shown in μ g/mL

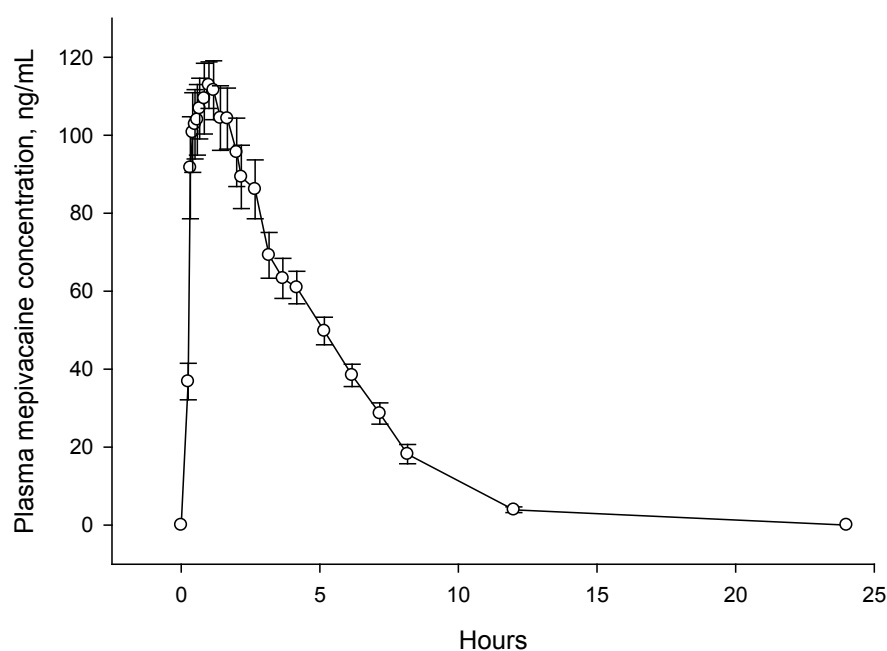


Figure 9.1 Plasma mepivacaine concentrations (mean $\pm$ se) after mepivacaine dosing (400 mg) in 12 horses

Table 9.2 Pharmacokinetic parameters for mepivacaine based on PK models fitted individually to observations from 12 horses

	Mean $\pm$ SD	Min	Max	Median
Peak plasma concentration ($C_{\max}$), $\mu\text{g/L}$	133.1 ± 31.9	79.2	182	128.5
Time to peak plasma concentration ($T_{\max}$), h	0.97 ± 0.49	0.30	1.85	1.00
Apparent volume of distribution central compartment (V), L	3358 ± 813	2252	5025	3233

Table 9.3 Pharmacokinetic parameters for mepivacaine in plasma based on a PK model fitted to 1000 replicate simulations

	Mean	Median	Range (2.5 th to 97.5 th percentiles)	Between subject variability
Apparent volume of distribution central compartment (V), L	3250	3270	2800 - 3870	-

Urine mepivacaine

The concentration of mepivacaine and its metabolites in urine is shown in Table 9.1 and Figure 9.2.

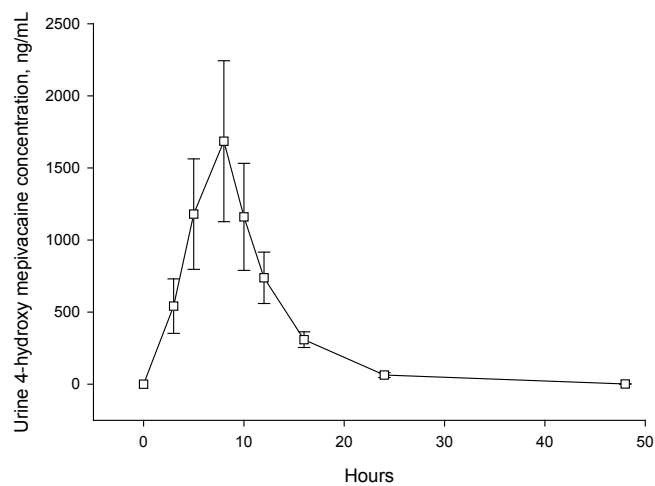
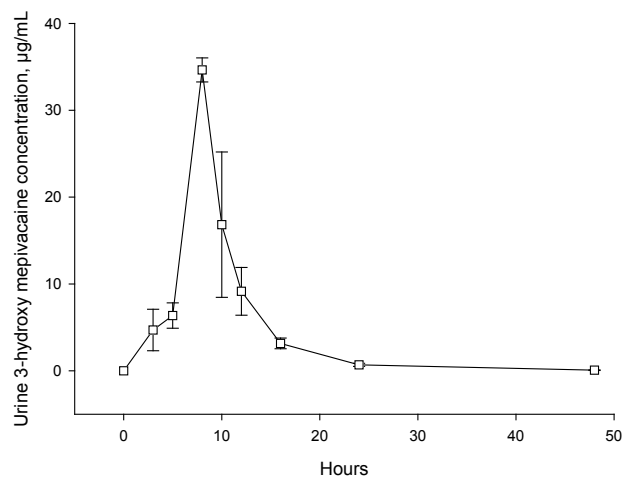
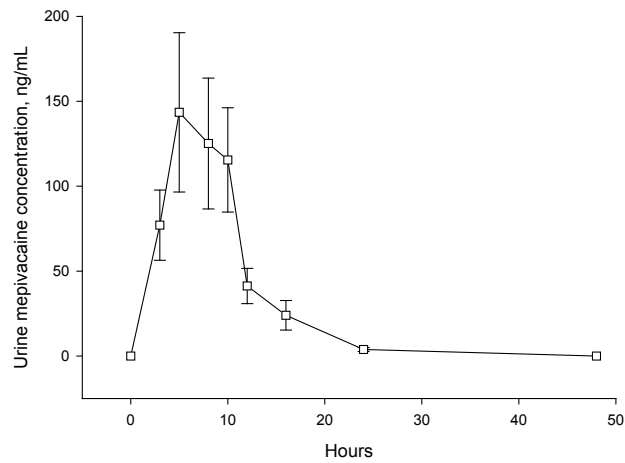


Figure 9.2 Urine concentrations of mepivacaine and two metabolites (mean $\pm$ se) after mepivacaine dosing (400 mg) in 12 horses. Note that concentrations of 3-hydroxy mepivacaine are shown in $\mu\text{g/mL}$

Mepivacaine concentrations in the urine peaked slightly earlier (5 h) than its metabolites (8 h) (Fig 9.2) and had fallen below the limit of accurate quantification in 5 of the 12 horses by 24 h. Mepivacaine was not detectable in the urine of any horse 48 h after administration.

The metabolite 3-hydroxy mepivacaine reached much higher concentrations in urine than mepivacaine (concentrations shown in $\mu\text{g/mL}$ in Table 9.1 and Figure 9.2) and its excretion was markedly slower. Although the mean concentration of 3-hydroxy-mepivacaine was below the limit of detection by 96 h, 3 of the 12 horses tested still exceeded this limit at that time. One horse had 3-hydroxy-mepivacaine concentration of 18.4 ng/mL 96 h after treatment, exceeding the limit of quantification by several-fold.

Relative to mepivacaine, 4-hydroxymepivacaine was also present in urine at high concentrations, although only 2 horses breached the detection level at 48 h and none of the horses showed detectable levels of this metabolite after 96 h.

Conclusions

The pharmacokinetics of mepivacaine in horses are complex and non-linear. Although the drug reaches peak plasma concentrations only 1 h after subcutaneous administration and is largely cleared from the circulation within 12 to 24 h, its major metabolite 3-hydroxy mepivacaine was detectable in a significant proportion of horses (25%) up to 96 h after mepivacaine administration. These data are currently being reviewed by the racing authorities with a view to setting an appropriate reporting level for the drug.

10 Prilocaine

Background

Prilocaine, *N*-(2-methylphenyl)-2-(propylamino) propanamide hydrochloride, is a local anaesthetic used for infiltration, intravenous regional anaesthesia and as a nerve block. It is available in Australia as Prilocaine Injection (Bomac) and Prilocaine 2% (Ceva Delvet).

As a member of the amide family, prilocaine is structurally and pharmacologically similar to lignocaine, with a similar time of onset and potency, but with less toxicity and a longer duration of action (eMIMS IVS Australia, 2011).

Prilocaine is known to undergo rapid metabolism in the liver and kidneys by enzymatic degradation (Covino, 1981), but otherwise there is very little information concerning the pharmacokinetics and metabolism of prilocaine in the horse. Some of the data presented here have already been reported by Glowacki et al. (in press).

Prilocaine administration

Prilocaine hydrochloride (20 mL SC/400 mg) was administered to 12 geldings (5 Thoroughbred, 7 Standardbred) housed at Charles Sturt University, NSW. The drug was given to the horses (mean $\pm$ se body weight 552 ± 13.4 kg) by subcutaneous injection as a single 400 mg dose in 20 mL of vehicle. This was equivalent to a dose rate of 0.60 to 0.84 mg/kg.

Blood and urine samples were collected before prilocaine administration and at intervals of decreasing frequency for up to 120 hours thereafter (Table 10.1).

Immediately after collection, blood samples were centrifuged ($2,800 \times g$ for 10 min), the plasma was divided into 2 portions and stored in plastic vials at -20°C . Urine samples were also divided into two aliquots and stored at -20°C . All samples were transported frozen to Racing Analytical Services, Vic., where they were analysed for the presence of prilocaine and its metabolites *o*-toluidine, 4-hydroxy-2-methylaniline and 6-hydroxy-2-methylaniline.

Extraction and analysis procedures

Calibration curves for prilocaine, *o*-toluidine, 4-hydroxy-2-methylaniline and 6-hydroxy-2-methylaniline in urine and blood were constructed over the range 10 to 1000 ng/mL using *o*-toluidine- d_9 and lignocaine as internal standards.

Urine samples were hydrolysed with β -glucuronidase, extracted using conditioned Plexa PCX solid phase extraction columns and eluted with ethyl acetate/dichloromethane/isopropanol with 2% ammonia.

Plasma samples were extracted after addition of ammonium acetate buffer, on conditioned Plexa PCX columns, as for urine.

Eluates were evaporated to dryness, reconstituted in 1% acetic acid/methanol (95:5) for analysis by LC-MS in full scan (50-500 Da), or by MS² using m/z 221.2, m/z 108.1 and m/z 124.1 as precursor ions for prilocaine, *o*-toluidine and both 4-hydroxy-2-methylamine and 6-hydroxy-2-methylaniline, respectively.

Pharmacokinetic modelling

The pharmacokinetics of Prilocaine were modelled at Charles Sturt University, NSW and The University of Pennsylvania, PA, using STATA Statistical Software 11.1 (Stata 2007) as described in the general methods section. PK data are presented as the mean $\pm$ SD. All other data are presented as mean $\pm$ se.

Results and discussion

Plasma prilocaine

Plasma concentrations of prilocaine and its major metabolite *o*-toluidine are shown in Table 10.1 and in Figures 10.1 and 10.2. The pharmacokinetic (PK) parameters for prilocaine in plasma are shown in Table 10.2.

According to the PK model, mean plasma concentrations of prilocaine were estimated to peak at 110 ng/mL approximately 35 min after injection. The PK analysis was only able to discern a single-phase system, and so the parameters listed in Table 10.2 represent the elimination kinetics of prilocaine over a 24 h period, after which the parent drug was no longer detectable in plasma. The mean terminal half-life for prilocaine elimination ($t_{1/2\beta}$) was 3 h 41 min.

In comparison, the highest mean plasma concentrations of *o*-toluidine (37 ng/mL) were observed 2 h post administration, and although the concentration fell steadily, *o*-toluidine was still detectable in plasma 24 h after prilocaine administration.

Table 10.1 Plasma concentrations of prilocaine (mean $\pm$ se) and toluidine following subcutaneous dosing with 400 mg prilocaine in 12 horses

Time	Prilocaine (ng/mL)	Toluidine (ng/mL)
0 h	0	0
5 min	20.9 $\pm$ 4.4	1.73 $\pm$ 0.32
10 min	60.0 $\pm$ 7.71	4.44 $\pm$ 0.83
20 min	91.8 $\pm$ 8.81	9.21 $\pm$ 1.23
30 min	105 $\pm$ 8.13	14.8 $\pm$ 2.19
40 min	95.3 $\pm$ 8.30	23.0 $\pm$ 4.48
50 min	89.6 $\pm$ 10.2	32.4 $\pm$ 5.54
1 h	73.1 $\pm$ 5.92	30.4 $\pm$ 3.86
1.5 h	48.3 $\pm$ 3.50	33.8 $\pm$ 4.34
2 h	37.3 $\pm$ 3.32	37.3 $\pm$ 5.37
2.5 h	27.7 $\pm$ 2.07	30.9 $\pm$ 4.51
3 h	24.0 $\pm$ 1.54	29.3 $\pm$ 3.61
4 h	17.0 $\pm$ 2.24	25.2 $\pm$ 3.39
6 h	14.1 $\pm$ 1.93	22.7 $\pm$ 1.87
8 h	7.80 $\pm$ 1.51	17.2 $\pm$ 2.50
12 h	1.64 $\pm$ 0.61	9.14 $\pm$ 1.79
24 h	0.24 $\pm$ 0.04	2.86 $\pm$ 0.39

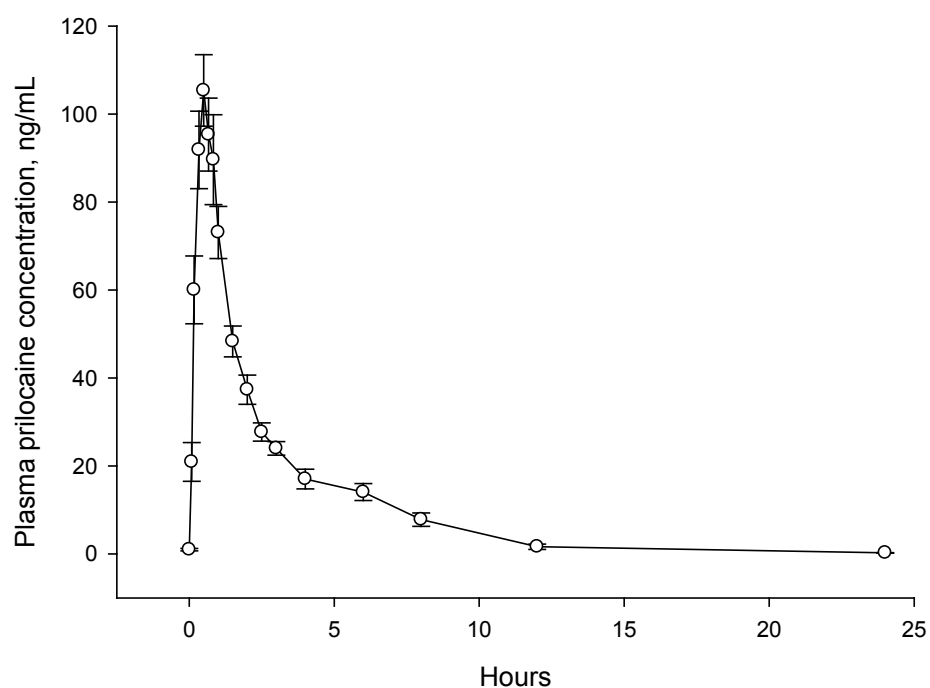


Figure 10.1 Plasma prilocaine concentrations (mean $\pm$ se) following subcutaneous dosing with 400 mg prilocaine in 12 horses

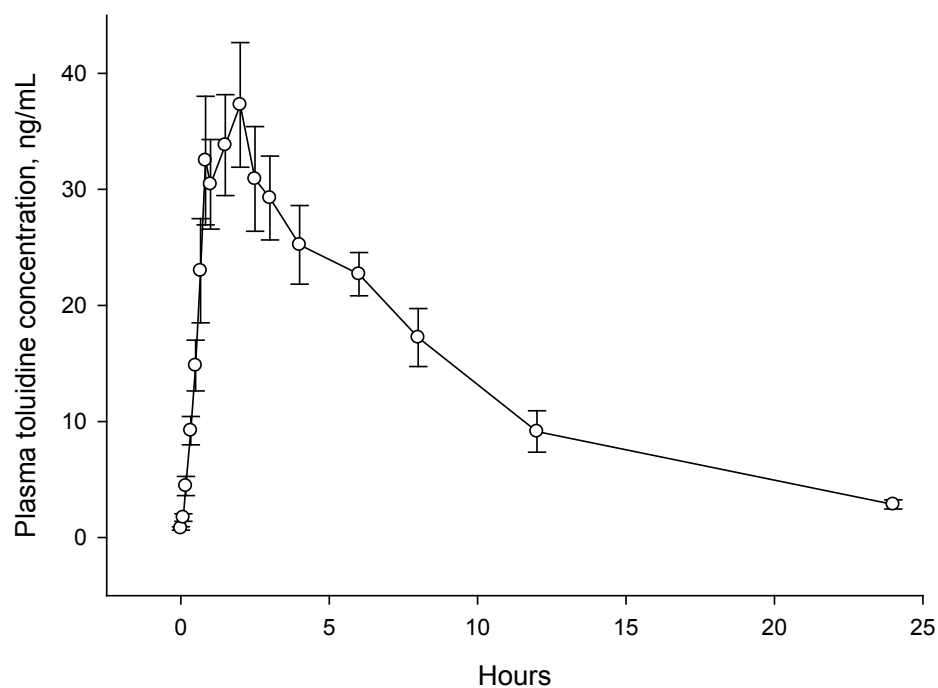


Figure 10.2 Plasma toluidine concentrations (mean $\pm$ se) following subcutaneous dosing with 400 mg prilocaine in 12 horses

Table 10.2 Pharmacokinetic parameters for prilocaine following subcutaneous dosing (400 mg) in 12 horses

	Mean $\pm$ SD	Min	Max	Median
Peak plasma concentration ($C_{\max}$), $\mu\text{g/L}$	110 $\pm$ 31.9	62.6	173	101
Time to peak plasma concentration ($T_{\max}$), h	0.58 $\pm$ 0.13	0.5	0.83	0.50
Apparent volume of distribution (V), L	1.77 $\pm$ 0.45	1.02	2.89	1.78
Terminal half-life ($T_{1/2}$ for β), h	3.68 $\pm$ 0.98	2.54	5.63	3.35

Urine prilocaine

With little information available regarding the metabolism of prilocaine in the horse, target compounds were proposed from metabolites identified in humans and based on the known metabolism of Lignocaine (Glowacki et al., in press).

The urinary metabolites of prilocaine consisted of *o*-toluidine, 4-hydroxy-2-methylaniline, 6-hydroxy-2-methylaniline, hydroxyprilocaine, despropylprilocaine and hydroxydespropylprilocaine. Urinary concentration of the first four substances are shown in Table 10.3 and in Figure 10.3

Table 10.3 Urine concentrations of prilocaine (mean $\pm$ se) and three metabolites following subcutaneous dosing with 400 mg prilocaine in 12 horses

Time	Prilocaine (ng/mL)	Toluidine (ng/mL)	4-hydroxy-2-methylaniline (ng/mL)	6-hydroxy-2-methylaniline (ng/mL)
0 h	0	0	0	0
2 h	41.5 $\pm$ 8.15	711 $\pm$ 148	20.3 $\pm$ 7.42	5.24 $\pm$ 1.72
4 h	299 $\pm$ 113	2037 $\pm$ 325	38.1 $\pm$ 10.1	19.6 $\pm$ 4.54
6 h	130 $\pm$ 16.2	1337 $\pm$ 172	63.5 $\pm$ 12.9	79.4 $\pm$ 1.45
8 h	83.4 $\pm$ 11.2	892 $\pm$ 59.1	40.5 $\pm$ 11.9	48.3 $\pm$ 1.45
12 h	60.6 $\pm$ 23.9	798 $\pm$ 55.1	22.0 $\pm$ 6.40	17.2 $\pm$ 8.28
24 h	1.71 $\pm$ 1.02	175 $\pm$ 28.8	3.52 $\pm$ 0.36	2.43 $\pm$ 0.88
32 h	0.15 $\pm$ 0.04	55.2 $\pm$ 6.62	3.23 $\pm$ 0.28	2.60 $\pm$ 0.80
48 h	0.06 $\pm$ 0.01	19.7 $\pm$ 2.64	3.09 $\pm$ 0.24	1.99 $\pm$ 0.58
72 h	0.06 $\pm$ 0.01	8.67 $\pm$ 0.79	2.93 $\pm$ 0.27	1.35 $\pm$ 0.41
96 h	0.06 $\pm$ 0.02	6.56 $\pm$ 0.50	2.88 $\pm$ 0.26	1.81 $\pm$ 0.53
120 h	0.08 $\pm$ 0.04	7.79 $\pm$ 1.01	3.12 $\pm$ 0.36	1.09 $\pm$ 0.29

Peak urinary concentrations of prilocaine (mean 299 ng/mL) were attained 4 h after administration. The three metabolites showed a broadly similar excretion profile, peaking 4 to 6 h after prilocaine injection, but *o*-toluidine in particular reached much higher concentrations (mean 2037 ng/mL). Accordingly, whereas prilocaine was detected in urine for up to 24 h, *o*-toluidine was detected in urine for up to 48 h.

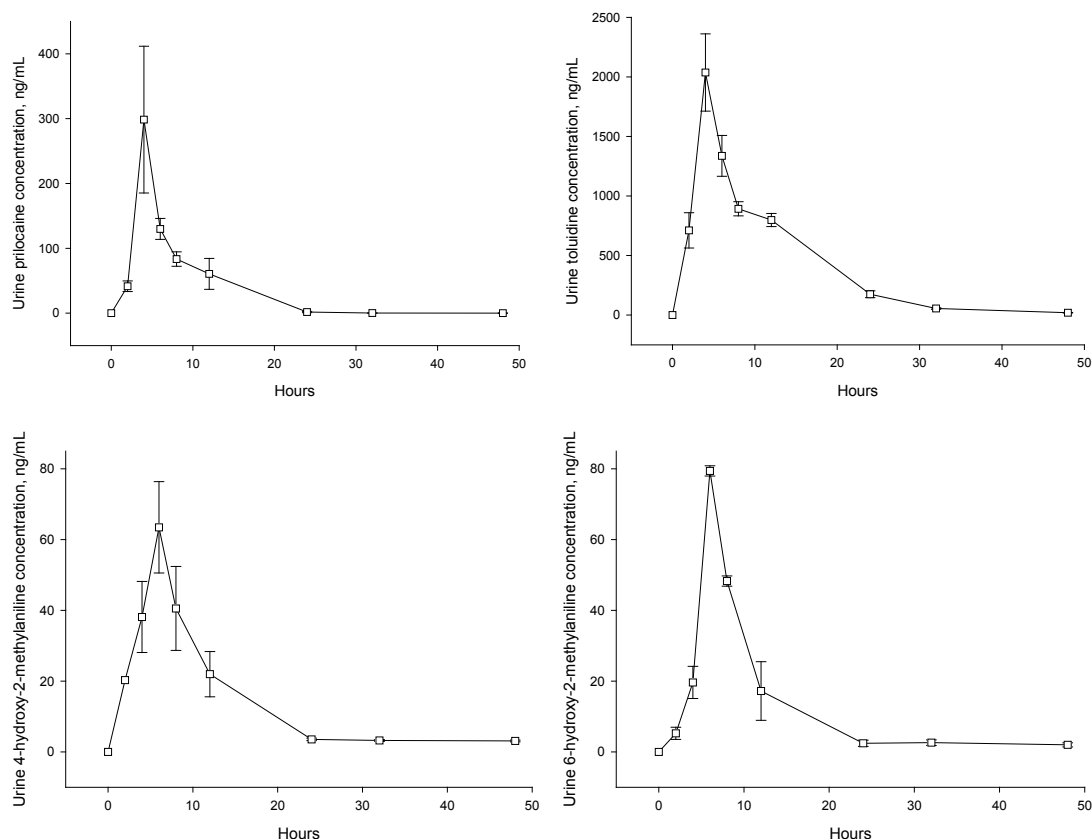


Figure 10.3 Concentrations of prilocaine (mean $\pm$ se) and three metabolites in urine following subcutaneous dosing with 400 mg prilocaine in 12 horses

Conclusions

Prilocaine has several advantages over other substances in its category, as a useful therapeutic agent for horses which require local anaesthesia.

We have reported the pharmacokinetics of prilocaine in plasma, which may assist veterinarians to time more accurately the drug administration and treatment procedures which need to be conducted under prilocaine anaesthesia.

Under the rules of racing, a horse may not compete if prilocaine or one of its metabolites is detectable in plasma or urine. Here we have demonstrated that *o*-toluidine is a major urinary metabolite of prilocaine, with two other metabolites produced in lesser amounts.

o-Toluidine is detectable in horse urine for up to 48 h after the administration of prilocaine at a standard therapeutic dose. These data are currently being reviewed by the racing authorities with a view to determining appropriate reporting levels consistent with the approach adopted by the European Horseracing Scientific Liaison Committee.

11 Detomidine

Background

Detomidine is an imidazoline derivative and a potent α_2 -adrenoreceptor agonist that has a central depressive action but is non-narcotic. It is used in horses as a sedative to enhance handling of difficult or nervous horses and to facilitate veterinary procedures such as farriery, minor surgery or radiography. Detomidine also has analgesic properties through central inhibition of pain. Accordingly, it is used to help manage painful conditions such as colic. Detomidine can also be used as a premedication for general anaesthesia (Valverde, 2010). It is widely used in equine practice and is frequently used in combination with other drugs for superior anaesthetic protocols (Hubbell et al., 2010).

Detomidine can be administered orally, IV, IM and as an epidural. The sedative effects are reported to be more pronounced following IV administration when compared to IM routes, however the duration of action may be reduced (Valverde, 2010). The drug is available in Australia: Dormosedan (Novartis Animal Health Australasia), Calmant Injection (Ranvet), Equisedan Vet Solution (Ausrichter), Detomo vet (Nature Vet) and Dozadine Injection (Axon Animal health). It is administered at a dose rate of 0.02 to 0.04 mg/kg IV for sedation/analgesia, but at a lower dose rate for epidural use (0.007 to 0.022 mg/kg (Valverde, 2010). When administered IV, detomidine has a rapid onset of action and a relatively small volume of distribution. Sedation is less pronounced following oral dosing (Kaukinen et al., 2011).

Alpha-2 receptor agonists can activate the receptors either pre or post-synaptically to mediate their effects, which include increases in arterial blood pressure and reduced heart rate (Valverde, 2010). Adverse effects of detomidine include ataxia, sweating, salivation, slight muscle tremors and piloerection. Alpha-2 agonists are also reported to reduce gastrointestinal motility and may induce ileus (Valverde, 2010).

Studies on the pharmacokinetics of detomidine and its metabolites, 3-hydroxy-detomidine (OH-detomidine) and detomidine 3-carboxylic acid (COOH-detomidine) in plasma have been conducted after intravenous and intramuscular injection (Salonen et al. 1989; Grimsrud et al. 2009; Hubbell et al. 2009). In urine, detomidine carboxylic acid was identified as the major urinary metabolite (Salonen et al. 1992). After IV administration of 30 μ g/kg detomidine, detomidine was last detected at 3 to 4 h post administration, OH-detomidine at 45 to 90 min and COOH-detomidine at 8 to 12 h (Grimsrud et al. 2009). After IM administration of 30 μ g/kg detomidine, detomidine was last detected at 5 to 6 h post administration, OH-detomidine at 6 h and COOH-detomidine at 8 to 12 h (Grimsrud et al. 2009). After a 80 μ g/kg dose of detomidine, most of the excretion of detomidine carboxylic acid occurred within 12 h of administration (Salonen et al. 1992).

Detomidine administration

Detomidine (Dormosedan, Novartis Animal Health Australasia) was administered as a single IV injection (0.04 mg/kg) to 12 geldings (5 Thoroughbred, 7 Standardbred, mean body weight 600 ± 11.3 kg) housed at Charles Sturt University, NSW.

Blood and urine samples were collected once prior to the detomidine injection, then at regular intervals of decreasing frequency up to 96 h after dosing (Table 11.1).

Immediately after collection, blood (20 mL) and urine (40 mL) samples were centrifuged (2 800 x g, 20 min). The plasma was divided into 2 portions and stored in plastic vials at -20°C . Urine samples were also divided into two portions and stored at -20°C . All samples were transported frozen to the

Racing Science Centre, Qld, where they were analysed for the presence of detomidine and its metabolites.

Extraction and analysis procedures

Urine samples were subjected to enzyme hydrolysis with β -Glucuronidase/aryl sulphatase (from *Helix pomatia*) before extraction. The samples were extracted using automated method employing solid-phase extraction cartridges (Waters Oasis WCX 60mg/3mL). Plasma samples underwent liquid/liquid extraction at pH 13-14 with di-isopropyl ether. All sample extracts were evaporated to dryness at 40°C under nitrogen and were then reconstituted in acetonitrile/water (50:50 v/v) for analysis by LC-MS-MS.

Pharmacokinetic modelling

The pharmacokinetics of detomidine in plasma were modelled at Charles Sturt University, NSW and The University of Pennsylvania, PA, using STATA Statistical Software 11.1 (Stata 2007) as described in the general methods section. PK data are presented as the mean $\pm$ SD. All other data are presented as mean $\pm$ se.

Results and discussion

Plasma detomidine

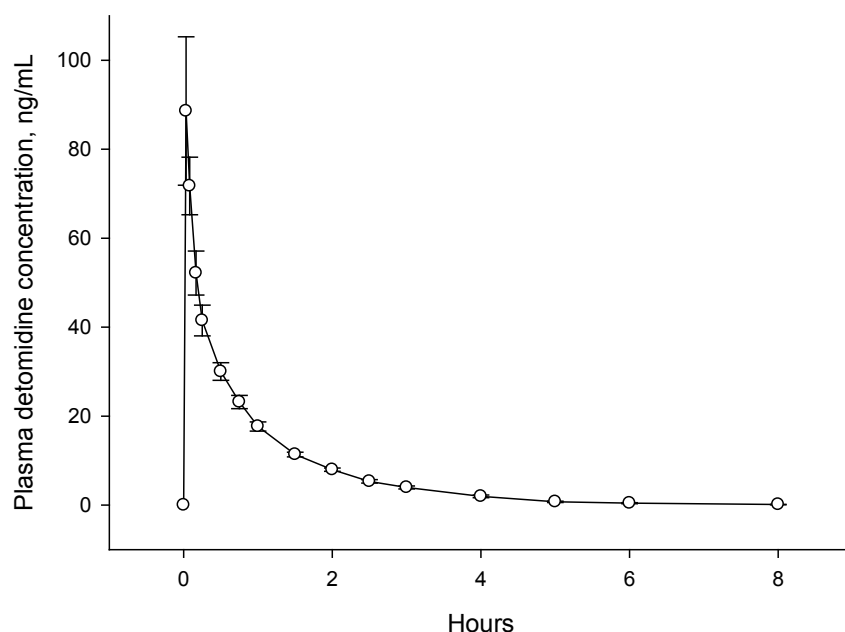


Figure 11.1 Plasma detomidine concentrations (mean $\pm$ se) following IV dosing (0.04 mg/kg) in 12 horses

Plasma concentrations of detomidine are shown in Fig. 11.1 and Table 11.1. Table 11.2 shows the pharmacokinetic (PK) analysis.

Table 11.1 Plasma and urine detomidine concentration (mean $\pm$ se) following IV dosing with detomidine (0.04 mg/kg) in 12 horses

Time	Plasma detomidine concentration (ng/mL)	Urine COOH-detomidine concentration (ng/mL)	Urine 3-OH-detomidine concentration (ng/mL)
0 h	0	0	0
5 min	88.6 $\pm$ 16.7	-	-
10 min	71.7 $\pm$ 6.47	-	-
15 min	41.5 $\pm$ 3.47	-	-
30 min	30.0 $\pm$ 1.97	-	-
45 min	23.2 $\pm$ 1.49	-	-
1 h	17.7 $\pm$ 1.04	-	-
1 h 30 min	11.4 $\pm$ 0.5	-	-
2 h	7.97 $\pm$ 0.38	90.7 $\pm$ 18.3	39.5 $\pm$ 10.5
2 h 30 min	5.34 $\pm$ 0.40	-	-
3 h	3.97 $\pm$ 0.34	-	-
4 h	1.99 $\pm$ 0.30	356 $\pm$ 59.2	130 $\pm$ 22.1
5 h	0.74 $\pm$ 0.16	-	-
6 h	0.44 $\pm$ 0.12	945 $\pm$ 172	349 $\pm$ 58.7
8 h	0.13 $\pm$ 0.05	566 $\pm$ 74.6	196 $\pm$ 48.6
12 h	0.05 $\pm$ 0.03	350 $\pm$ 32.1	119 $\pm$ 16.6
24 h	0.02 $\pm$ 0.02	37.7 $\pm$ 6.97	9.19 $\pm$ 2.54
32 h	0	6.94 $\pm$ 1.94	2.18 $\pm$ 0.61
48 h	0	0.53 $\pm$ 0.23	0.19 $\pm$ 0.05
72 h	0	0	0.13 $\pm$ 0.06
96 h	0	0	0

Table 11.2 Pharmacokinetic parameters following IV dosing with detomidine (0.04 mg/kg) in 12 horses

	Mean $\pm$ SD	Min	Max	Median
Peak plasma concentration ($C_{\max}$), μ g/L	99.2 $\pm$ 52.3	22.1	227	88.9
Time to peak plasma concentration ($T_{\max}$), min	3.0 $\pm$ 0.25	2.0	5.0	2.0
Apparent volume of distribution (V), L	0.55 $\pm$ 0.42	0.18	1.81	0.45
Terminal half-life ($T_{1/2}$ for β), h	1.19 $\pm$ 0.48	0.5	2.12	1.10

According to the PK model, detomidine concentrations would have peaked in the plasma after only 3 min, which is consistent with IV dosing. The elimination half life ($t_{1/2\beta}$) of detomidine was short (71 min) and the pharmacokinetics of the drug were consistent with a 1-compartment model (Table 11.2).

The last time-point at which detomidine could be reliably detected in the plasma of all horses was 3 h after administration. Nevertheless, detomidine was detectable in 10 of the 12 horses 4 h after administration, and in 1 horse for up to 6 h. None of the horses had plasma detomidine concentrations above the level of accurate quantification 8 h after administration.

Urine detomidine

Urine concentrations of the detomidine metabolites (COOH-detomidine and 3-OH-detomidine) are shown in Table 11.1 and Figure 11.2.

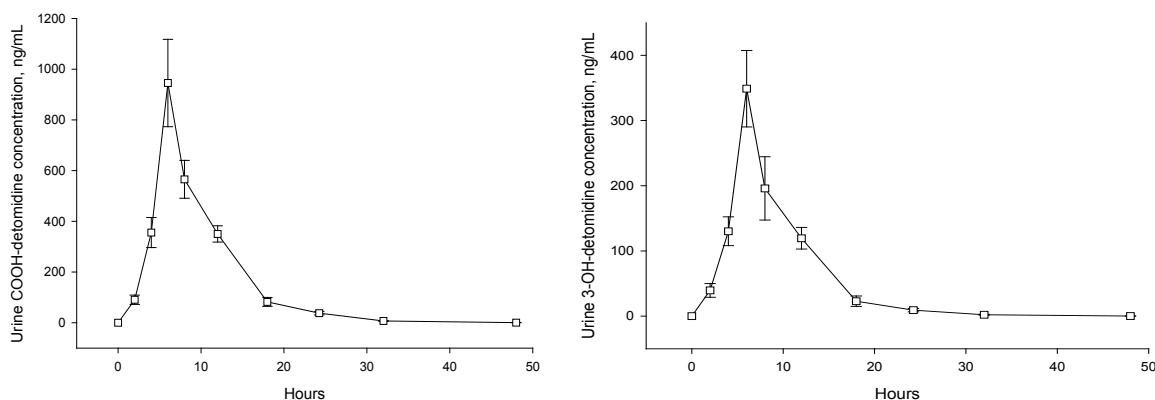


Figure 11.2 Urine concentrations of COOH-detomidine and 3-OH-detomidine (mean $\pm$ se) following IV dosing of detomidine (0.04 mg/kg) in 12 horses

The urinary excretion pattern of the two metabolites was very similar (Fig 11.2). The mean time for both metabolites to reach peak concentrations in urine was estimated to be 6 h 12 min after detomidine administration. The mean ($\pm$ se) size of the peak was estimated to be 979 ± 166 ng/mL for COOH-detomidine and 442 ± 67 ng/mL for 3-OH-detomidine.

Both metabolites were detectable in 11 out of 12 horses 24 h after detomidine administration. By 32 h COOH-detomidine and 3-OH-detomidine were detectable in 4 and 5 horses, respectively. The latter metabolite was still detected in 1 horse after 48 h, and none of the horses returned a positive test after 72 h.

Conclusions

Detomidine is a valuable therapeutic agent as a fast-acting sedative (when given IV) which largely disappears from the blood circulation within 5 h and from urine (in the form of its metabolites) within 48 h. However, in one horse detomidine was detectable at very low concentrations in plasma for up to 6 h, while its metabolite 3-OH-detomidine, was also detectable in urine for up to 48 h.

The significance of these data are currently being reviewed by the racing authorities with a view to setting appropriate reporting levels for detomidine.

12 Acepromazine

Background

Acepromazine (ACP, also known as acetylpromazine) is a commonly used phenothiazine tranquilizer given to reduce excitement and stress during various veterinary procedures, and as pre-anaesthetic agent in barbiturate anaesthesia (Posner and Burns, 2009). It is also administered to some horses during training if they are nervous or difficult to handle, and is sometimes used during transport and confinement to relieve anxiety.

Acepromazine is available in Australia: ACP 10 injection (Ceva Delvet), Acemav Injection (Mavlab) and Ilium Acepril-10 injection, Troy Laboratories. The usual route of administration is either IV or IM, although an oral gel formulation is also available; Ilium Sedaquin gel (Troy Laboratories) and Sedazine-ACP paste (Vetsearch International).

Following administration, large amounts of ACP become bound to plasma proteins and red blood cells (erythrocytes). Free ACP is converted rapidly to a major metabolite known as 2-(1-hydroxyethyl) promazine sulphoxide (HEPS) (Weir and Sanford, 1972), which is excreted unconjugated in the urine. Apart from the influence of a rapid rate of metabolism, the concentration of ACP in blood and urine can vary markedly depending on administration of a single or multiple doses, and the exercise status of the horse (Chou et al., 2002).

Pharmacokinetic (PK) studies of ACP have been conducted by others, but previous experiments have used only four to six horses and showed a large variation between individuals (Ballard et al., 1982; Hashem and Keller, 1993; Marroum et al., 1994). The aim of the present study was to produce more accurate PK data and to estimate excretion times more reliably for ACP and HEPS.

Acepromazine administration

Acepromazine was administered to 12 mature geldings (4 Thoroughbreds and 8 Standardbreds; mean age 8.5 ± 0.8 years old) housed at Charles Sturt University, NSW. The drug was given to the horses (mean $\pm$ se body weight 551 ± 14 kg) in the form acepromazine maleate (ACP 10, Delvet NSW, 10 mg/mL) by IV injection, at a fixed dose of 3 mL per horse, which delivered 30 mg of ACP. This was equivalent to a dose rate of 0.045 to 0.063 mg/kg.

Blood and urine samples were collected before ACP administration and at intervals of decreasing frequency for up to 144 h after drug administration. Blood samples were collected into 10 mL lithium heparin vacutainers and centrifuged at 4 °C (20 min; 2,800 x g). Plasma was harvested and plasma and urine samples were stored at -20 °C until analysed.

All samples were transported frozen to the Australian Racing Forensic Laboratories, NSW, where they were analysed for the presence of ACP and its metabolite, HEPS.

Extraction and analysis procedures

Aliquots of plasma were spiked with propionylpromazine hydrochloride then diluted with methanol and acetic acid before being centrifuged to precipitate proteinaceous material. ACP and HEPS were extracted from the supernatant fractions using IST Isolute HCX solid phase extraction cartridges which had previously been conditioned with methanol and acetic acid. The cartridges were washed with acetic acid and methanol, then dried and eluted with ethyl acetate:methanol:ammonium

hydroxide. The eluates were evaporated to dryness at 60°C under a stream of nitrogen, then were reconstituted in isopropanol and ammonium acetate for LC-MS analysis.

Aliquots of urine were also spiked with propionylpromazine hydrochloride, then were diluted with acetic acid and centrifuged to precipitate particulate material. The supernatant fractions were decanted, then extracted and reconstituted for LC-MS analysis as described above for the plasma samples.

Samples were analysed using a Waters Acquity UPLC system equipped with a Waters Acquity UPLC BEH C18 analytical column and Phenomenex Security-Guard C18 guard column interfaced to an Applied Biosystems 4000 Q-Trap mass spectrometer.

The specificity of the method was assessed by analysing 12 blank equine plasma and 12 blank equine urine samples. No significant matrix interference was observed at the retention times of the analytes of interest. The calibration curves for ACP in both plasma and urine were linear over the full calibration ranges and correlation coefficients were 0.9889 or greater were obtained in both matrices. The limit of quantification (LOQ) in both matrices was 1 ng/mL. The limit of detection (LOD), based on a signal to noise ratio greater than 3, was less than 1 ng/mL in all cases.

Pharmacokinetic modelling

The pharmacokinetics of Acepromazine were modelled at Charles Sturt University, NSW and The University of Pennsylvania, PA, using WinSAAM software (<http://www.winsaam.com>, University of Pennsylvania) and STATA Statistical Software 11.1 (Stata, 2007) as described in the general methods section.

The urine-to-plasma (U/P) ratio for HEPS was determined by regressing plasma HEPS on urine HEPS and dividing the urine concentration by the plasma concentration of the metabolite at the time points 2, 4, 6, 8, 12 and 24 h, then calculating the mean of the 12 horses at each time point.

PK data are presented as the mean $\pm$ SD. All other data are presented as mean $\pm$ se.

Results and discussion

Plasma acepromazine

Concentrations of ACP and HEPS are shown in Table 12.1 and Figure 12.1. ACP levels reached a peak of 33.8 ng/mL in plasma after only 5 min, then fell rapidly, such that on average, they were below the limit of accurate quantification (i.e. <1 ng/mL) within 2 h. This indicates a rapid conversion of ACP to its metabolite HEPS. The large standard error bars in Figure 12.1 show the wide variation observed between horses in the metabolism of ACP, particularly during the elimination phase.

When the ACP data were modelled they showed a close fit to a biphasic equation, hence the kinetics of the drug were calculated by a 2-compartment open model as described by Hasham and Keller (1993). A summary of the PK parameters for ACP is given in Table 12.2. The half-life of distribution ($t_{1/2\alpha}$) was 0.15 h, while the mean half-life of elimination was ($t_{1/2\beta}$) 119 min. This result is consistent with previous studies which report a $t_{1/2\beta}$ ranging from 50 to 185 min (Ballard et al., 1982; Hashem and Keller, 1993; Marroum et al., 1994).

The clearance rate of ACP was estimated to be 4.9 L/h/kg, while clearance rates of between 2.5 and 3 L/h/kg have been reported elsewhere (Ballard et al., 1982; Hashem and Keller, 1993; Marroum et al., 1994).

It should be noted that only about 10% of the ACP administered in the present study could be accounted for, consistent with previous observations that the drug is bound extensively to erythrocytes (Ballard et al., 1982).

Table 12.1 Plasma and urine concentration of acepromazine (ACP) and 2-(1-hydroxyethyl) promazine (HEPS) (mean $\pm$ se) following intravenous dosing with 30 mg acepromazine in 12 horses

Time	Plasma ACP (ng/mL)	Plasma HEPS (ng/mL)	Urine ACP (ng/mL)	Urine HEPS (ng/ml)
0 h	0	0	0.23 $\pm$ 0.11	1.19 $\pm$ 0.34
5 min	33.8 $\pm$ 9.80	5.18 $\pm$ 0.51	-	-
10 min	19.6 $\pm$ 9.56	3.89 $\pm$ 0.44	-	-
20 min	6.93 $\pm$ 2.07	3.70 $\pm$ 0.27	-	-
40 min	4.40 $\pm$ 1.18	3.90 $\pm$ 0.32	-	-
1 h	2.83 $\pm$ 0.38	4.11 $\pm$ 0.31	-	-
1.5 h	1.80 $\pm$ 0.16	3.56 $\pm$ 0.36	-	-
2 h	0.95 $\pm$ 0.16	3.20 $\pm$ 0.26	1.02 $\pm$ 0.26	585 $\pm$ 169
2.5 h	0.74 $\pm$ 0.11	3.01 $\pm$ 0.33	-	-
3 h	0.59 $\pm$ 0.10	2.93 $\pm$ 0.36	-	-
4 h	0.52 $\pm$ 0.13	3.03 $\pm$ 0.29	1.47 $\pm$ 0.47	954 $\pm$ 276
6 h	0.30 $\pm$ 0.04	2.32 $\pm$ 0.24	0.37 $\pm$ 0.20	459 $\pm$ 133
8 h	0.33 $\pm$ 0.05	1.97 $\pm$ 0.20	0.21 $\pm$ 0.09	330 $\pm$ 95.3
12 h	0.35 $\pm$ 0.07	1.38 $\pm$ 0.13	0.29 $\pm$ 0.15	236 $\pm$ 68.2
16 h	-	-	0.20 $\pm$ 0.07	107 $\pm$ 30.9
24 h	0.33 $\pm$ 0.06	0.42 $\pm$ 0.04	0.25 $\pm$ 0.17	83.6 $\pm$ 24.2
28 h	-	-	0.15 $\pm$ 0.07	45.6 $\pm$ 13.2
32 h	0.23 $\pm$ 0.05	0.27 $\pm$ 0.03	0.42 $\pm$ 0.16	28.9 $\pm$ 8.39
36 h	-	-	0.30 $\pm$ 0.13	24.2 $\pm$ 7.00
40 h	-	-	0.36 $\pm$ 0.14	13.7 $\pm$ 3.97
48 h	0.25 $\pm$ 0.05	0.13 $\pm$ 0.01	0.26 $\pm$ 0.12	9.26 $\pm$ 2.68
72 h	0.33 $\pm$ 0.10	0.09 $\pm$ 0.01	0.20 $\pm$ 0.11	2.47 $\pm$ 0.71
96 h	0.31 $\pm$ 0.08	0.11 $\pm$ 0.01	0.29 $\pm$ 0.14	1.65 $\pm$ 0.48
120 h	0.26 $\pm$ 0.02	0.09 $\pm$ 0.01	0.26 $\pm$ 0.12	1.47 $\pm$ 0.43
144 h	0.33 $\pm$ 0.07	0.11 $\pm$ 0.01	0.20 $\pm$ 0.12	1.29 $\pm$ 0.37

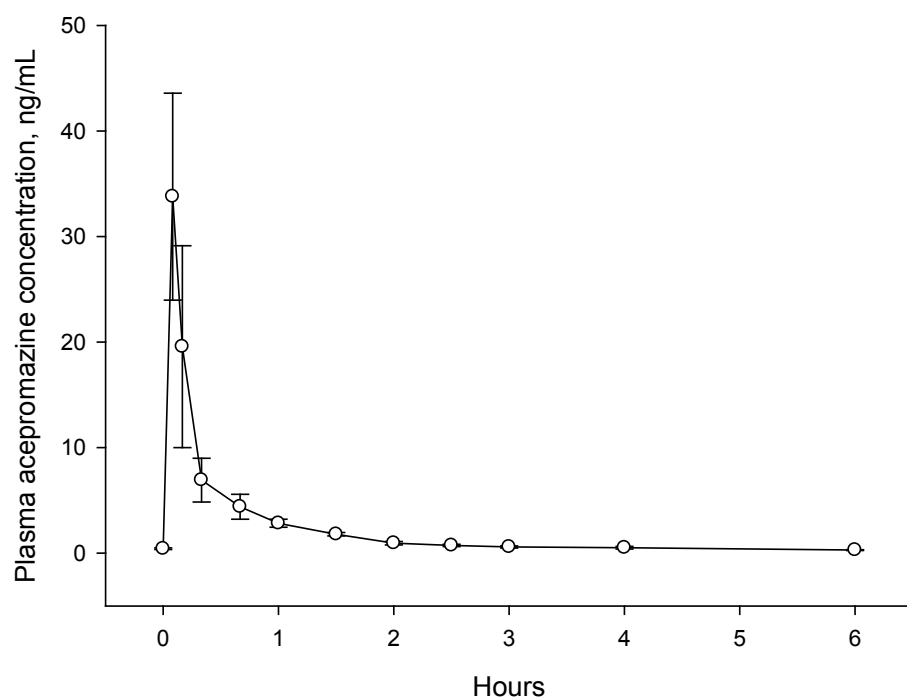


Figure 12.1 Plasma acepromazine concentrations (mean $\pm$ se) after intravenous dosing with 30 mg acepromazine in 12 horses

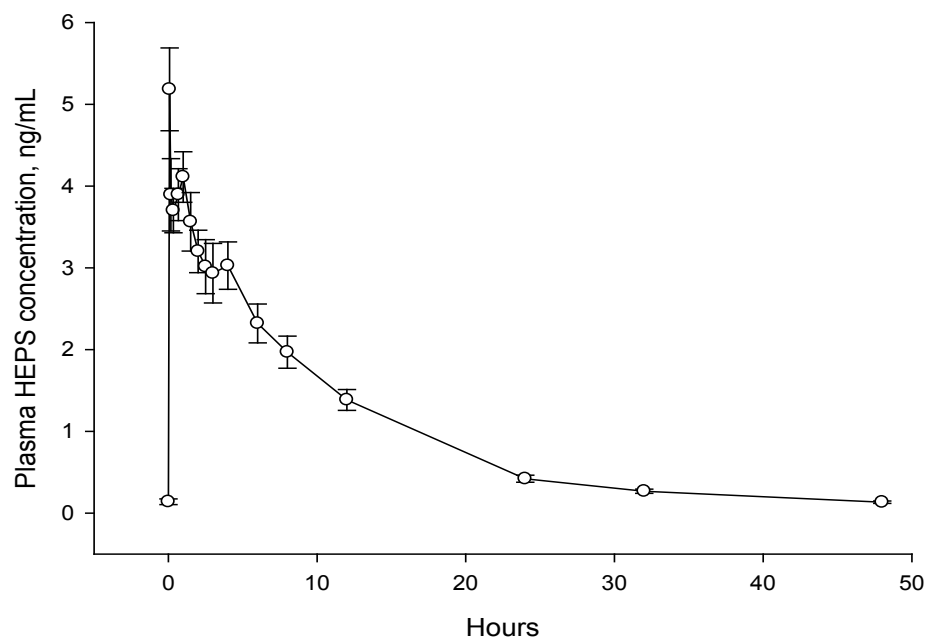


Figure 12.2 Plasma 2-(1-hydroxyethyl) promazine sulfoxide (HEPS) concentrations (mean $\pm$ se) after intravenous dosing with 30 mg acepromazine in 12 horses

Table 12.2 Pharmacokinetic parameters for plasma acepromazine after intravenous dosing (30 mg) in 12 horses

	Mean $\pm$ SD	Min	Max	Median
Peak plasma concentration ($C_{\max}$), $\mu\text{g/L}$	33.8 ± 33.9	12.7	137	23.2
Time to peak plasma concentration ($T_{\max}$), min	5 ± 0	5	5	5
Apparent volume of distribution (V), L	2.18 ± 2.82	0.09	10.2	1.35
Compartment 1				
Terminal half-life ($T_{1/2}$ for α), h	0.15	0.04	0.42	0.08
Compartment 2				
Terminal half-life ($T_{1/2}$ for β), h	1.98 ± 1.66	0.53	23.0	1.61

Plasma HEPS

The plot of HEPS concentration in plasma showed an early chaotic phase which could not be modelled (Figure 12.3). However, the data resolved into slopes that were similar for each horse once the pattern settled down.

As HEPS is the product of rapid metabolism of ACP, it was assumed that the early kinetics of HEPS would be similar to those of ACP for this distribution phase. In fact, a strong relationship between the peak plasma HEPS concentration and the y-intercept of the kinetic model was observed, thus allowing valid interpretation of the elimination phase. This relationship allowed for the PK analysis of plasma HEPS to be conducted for a single compartment, enabling simple linear solutions.

A summary of the PK parameters for HEPS in plasma is shown in Table 12.3. There was a consistent elimination of HEPS across all horses of 0.095 /h, despite the early chaos. The a half-life of elimination ($t_{1/2\gamma}$) was estimated to be 7.64 ± 0.34 h for HEPS, which was significantly longer ($P < 0.05$) than the half-life for ACP (1.98 h). The clearance rate (Cl) for HEPS was 1.34 ± 0.10 L/kg/h and the volume of distribution (Vd) was 2.30 ± 0.98 L/kg. The early chaotic emergence of HEPS into the system did compromise the determination of Vd and Cl for this metabolite. However, reconciling Cl with the conversion fraction does support these values.

This study did not measure pharmacological effects of ACP so no conclusions could be drawn on the sedative effects of ACP based on HEPS plasma concentrations, which are reported to last anywhere from 2 h (Marroum et al. 1994) to 6 h (Hasham and Keller 1993) post-administration. However, Marroum et al. (1994) suggested that, as peak observable pharmacological effects did not correlate with peak plasma concentrations of ACP, the effects of the drug may be mediated by an active metabolite. This metabolite would need to be present in sufficient amounts to exert noticeable sedative effects. If one considers the half-life of ACP (approx 2 h) compared to HEPS (approx 8 h), it is conceivable that HEPS may play a role to play in the sedative effect of ACP. The direct administration of HEPS to the horse would be necessary to confirm this hypothesis

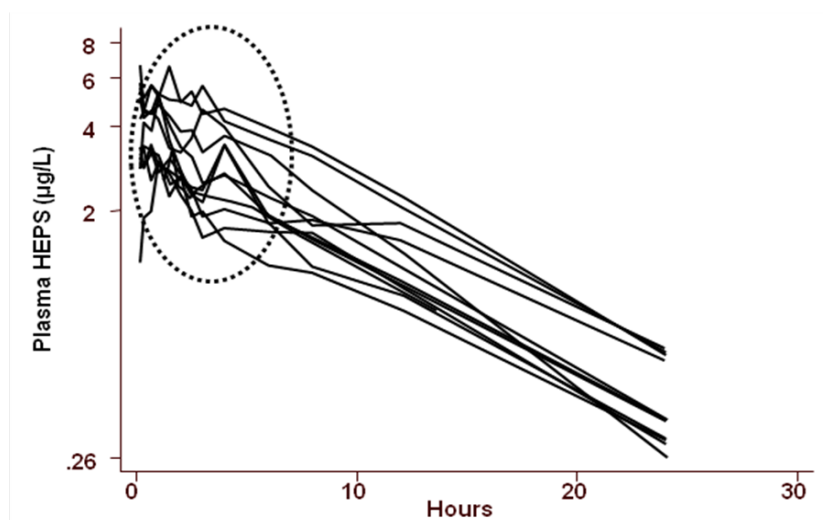


Figure 12.3 Semi-logarithmic plot of plasma 2-(1-hydroxyethyl) promazine sulfoxide (HEPS) concentration versus time in 12 horses administered 30 mg acepromazine. The early chaotic appearance of plasma HEPS is highlighted by a dotted line. However, similar slopes are observed as time passes and metabolism becomes more stable

Table 12.3 Pharmacokinetic parameters for the metabolite 2-(1-hydroxyethyl) promazine sulfoxide (HEPS) following the intravenous administration of acepromazine (30 mg) to 12 horses

	Mean $\pm$ SD	Min	Max	Median
Peak plasma concentration ($C_{\max}$), $\mu\text{g/L}$	5.57 ± 1.55	2.71	7.81	5.72
Time to peak plasma concentration ($T_{\max}$), min	5.52 ± 0.1	5	92	7.5
Apparent volume of distribution (V), L/kg	2.30 ± 3.38	0.58	12.6	1.10
Terminal half-life ($T_{1/2}$ for β), h	7.64 ± 1.18	5.86	10.6	7.51

Urine Acepromazine and HEPS

Concentrations of ACP and HEPS in urine are shown in Table 12.1 and in Figures 12.4 and 12.5. The highest concentration of ACP in urine seen in any horse was only 5.23 ng/mL, at 4 h post ACP administration. The fm was calculated at 0.99 ± 0.01 , meaning that almost all the available ACP was metabolised to HEPS in the body.

Chou et al. (1998) reported a much higher peak concentration of ACP of in urine (49.2 ng/mL), also 4 h after the administration of ACP at a similar dose rate (25 mg), but by intramuscular injection. It is likely that the different route of administration used in that study resulted in slower and less complete metabolism of ACP, allowing more of the drug to be excreted unaltered by the kidneys.

In the present study, peak concentrations of HEPS in urine were also seen 4 h after the administration of ACP. Ninety eight percent of HEPS was excreted in the first 48 h, but as concentrations of the metabolite were relatively high, the substance could be reliably detected in urine for up to 144 h.

The high urinary concentrations of HEPS were reflected by high U/P concentration ratios which were calculated from 2 h to 24 h. These ratios ranged from 170 to 321 depending on time elapsed, averaging 177.62 ± 0.77 across all time points.

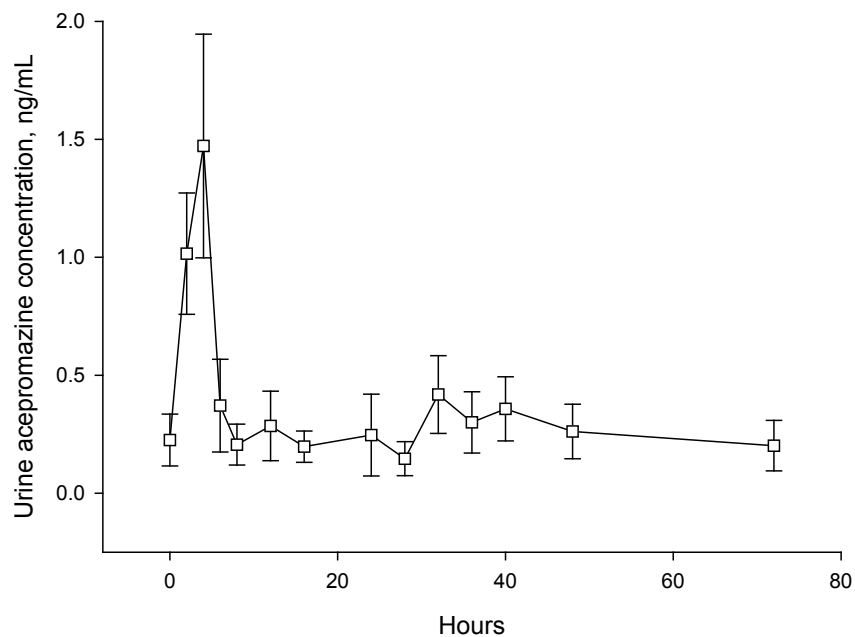


Figure 12.4 Urine concentrations of acepromazine (mean $\pm$ se) after intravenous dosing with 30 mg acepromazine in 12 horses

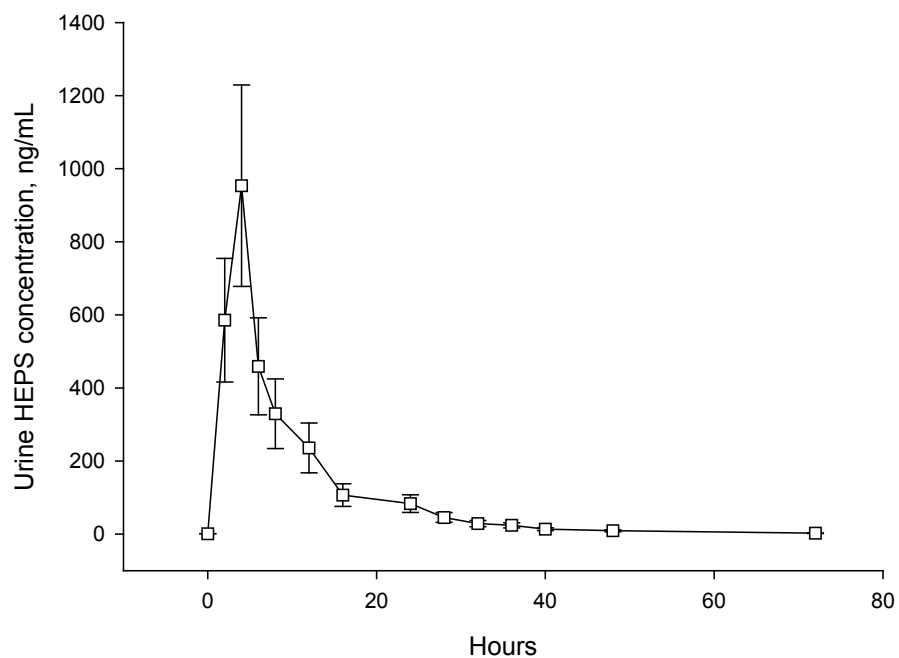


Figure 12.5 Urine 2-(1-hydroxyethyl) promazine sulfoxide (HEPS) concentrations (mean $\pm$ se) after intravenous dosing with 30 mg acepromazine in 12 horses

Conclusions

The concentration of free ACP in plasma peaks within 5 min of drug administration and considerable variation is observed between horses as the tranquilizer is rapidly converted to the metabolite HEPS.

However, as much as 90% of the ACP remains bound to plasma proteins and erythrocytes, so there is a slow elimination of ACP and HEPS from the body. This translates to much longer detection times for the metabolite, which can be reliably measured in urine for up to 144 h.

The prolonged detection time in both plasma and urine makes HEPS a more suitable target for forensic analyses to investigate whether ACP has been administered within a forbidden timeframe close to competition.

A consistent relationship between HEPS concentrations in urine and plasma was demonstrated in this study. The value of the U/P ratio is that should a positive result be obtained from a urine sample, by applying the U/P ratio, the plasma concentration of HEPS can be estimated. In turn, the linear relationship between plasma HEPS and ACP means the time of administration of the ACP could be estimated, which may be of value in any investigation of a positive result.

The data generated in this study are currently being reviewed by the racing authorities with a view to determining appropriate reporting levels consistent with the approach adopted by the European Horseracing Scientific Liaison Committee.

Meanwhile, a novel approach has been developed to estimating withholding times for ACP, taking into account the risk of breaching a detection threshold at any given time point after ACP administration. This new approach, employing Bayesian statistics, is described in Chapter 13.

13 A Bayesian probabilistic approach to determining detection and withholding times for acepromazine

Introduction

This chapter describes a modified and extended approach to the modelling of pharmacokinetic (PK) data for acepromazine, illustrating the application of Bayesian statistics, the measurement of uncertainty around PK parameters in a horse population, and the use of risk analysis in gauging appropriate withholding times for therapeutic substances.

As discussed in Chapter 12, acepromazine (ACP) is a phenothiazine tranquilizer commonly used in horses. After IV administration the drug is bound extensively by plasma proteins (>99%) and has a large volume of distribution (Ballard et al., 1982). Acepromazine undergoes extensive metabolism in the liver, producing both conjugated and unconjugated metabolites. A small quantity of unmetabolised ACP is eliminated in the urine, together with a larger quantity of its principle metabolite 2-(1-hydroxyethyl) promazine sulphoxide (HEPS).

Although the individual, horse-specific pharmacokinetics for ACP have been studied previously (Ballard et al., 1982; Hashem and Keller, 1993; Marroum et al., 1994), to our knowledge, no PK approach has been applied to HEPS, no population analysis has been conducted, and no risk analysis has been applied concerning appropriate detection or withholding times.

In the present study we developed an understanding of the PK and renal excretion of HEPS at a population level through the implementation of a Bayesian hierarchical model (BHM). Covariates were available including breed, body weight and age, and these were explored as potential effects to minimize unexplained between-subject variability. Also, as each sample was analysed in duplicate, the degree of measurement error was examined as another potential source of variability. Once a simple statistical model had been developed, simulation techniques were used to estimate detection times in plasma and urine.

Methods

Data were collected from 12 geldings after the administration of acepromazine maleate equivalent to 30 mg of ACP (0.045 to 0.063 mg/kg BW). Details of the horses are shown in Table 13.1. The mean ($\pm$ se) body weight of the horses was 551 ± 14 kg and the mean ($\pm$ se) age was 8.5 ± 0.8 years.

ACP and HEPS concentration-time data were derived from the chemical analysis of blood and urine samples collected at frequent intervals as described in Chapter 12.

A full conditional hierarchical Bayesian analysis was undertaken using WinBUGS software (Lunn et al., 2000), where Markov chain Monte Carlo (MCMC) techniques are used to sample from the posterior distribution of estimable parameters. Reviews which illustrate the use of Bayesian modelling of PK data have been published previously (Duffull et al., 2005; Lunn et al., 2002).

In the present analysis, two MCMC chains were run simultaneously, each with different initial estimates for the designated parameters. Convergence of the chains to the stationary distributions was assessed using the Gelman and Rubin convergence diagnostic (Kass et al., 1998), with a value of <1.1 taken to indicate that a stationary distribution had been reached. In addition, the trace history for

each parameter was plotted, to ensure the ‘fuzzy caterpillar’ was observed, as described by Lunn et al. (1999).

The two MCMC chains were run for 20,000 samples with the first 10,000 being discarded as a ‘burn-in’. If the convergence criteria were met, the two chains were pooled to provide samples from the stationary/posterior distribution. All inferences were drawn based on this distribution.

Table 13.1 Details of 12 geldings used to examine the pharmacokinetics of acepromazine (ACP)

Horse ID	Weight (kg)	Age (yrs)	Breed	Dose ACP administered (mg/kg BW)
1	570	11	Thoroughbred	0.053
2	540	15	Thoroughbred	0.056
3	670	10	Thoroughbred	0.045
4	544	9	Standardbred	0.055
5	550	8	Thoroughbred	0.055
6	574	9	Standardbred	0.052
7	538	7	Standardbred	0.056
8	566	8	Standardbred	0.053
9	478	4	Standardbred	0.063
10	552	5	Standardbred	0.054
11	474	7	Standardbred	0.063
12	560	9	Standardbred	0.054

The parent and metabolite model

A conceptual model can be constructed to illustrate the conversion of the parent drug (ACP) to its metabolite (HEPS), and the elimination of both substances from the body. The full parent-metabolite model based on mass balance is given in Figure 1.

A fraction of the parent is either converted to the metabolite (FPM), excreted renally (FEP), or eliminated non-renally ($1 - \text{FPM} - \text{FEP}$). In the second case, the triangle represents the bladder, and the parent will continue to accumulate here until the horse empties the bladder. For the fraction of the parent that is converted to the metabolite, a fraction of M is either excreted renally (FEM) or non-renally ($1 - \text{FEM}$). In this first case, the metabolite accumulates in the bladder (represented by a triangle) until the horse empties the bladder. The q symbols represent rate constants for movement between compartments.

While the full 5-compartment parent-metabolite model illustrates the various conceptual pathways for ACP, in practice a simplified model was used. Given the half-life of ACP was considerably shorter than that of HEPS, it was apparent that the kinetics of the metabolite were not being driven by the parent. In such cases, it should be sufficient to model the metabolite alone, particularly given we are interested in estimating detection times for the metabolite. Notably, this is a key assumption in our approach, and further research would be required to determine if this biased our analysis. The simplified model is shown in Figure 13.2.

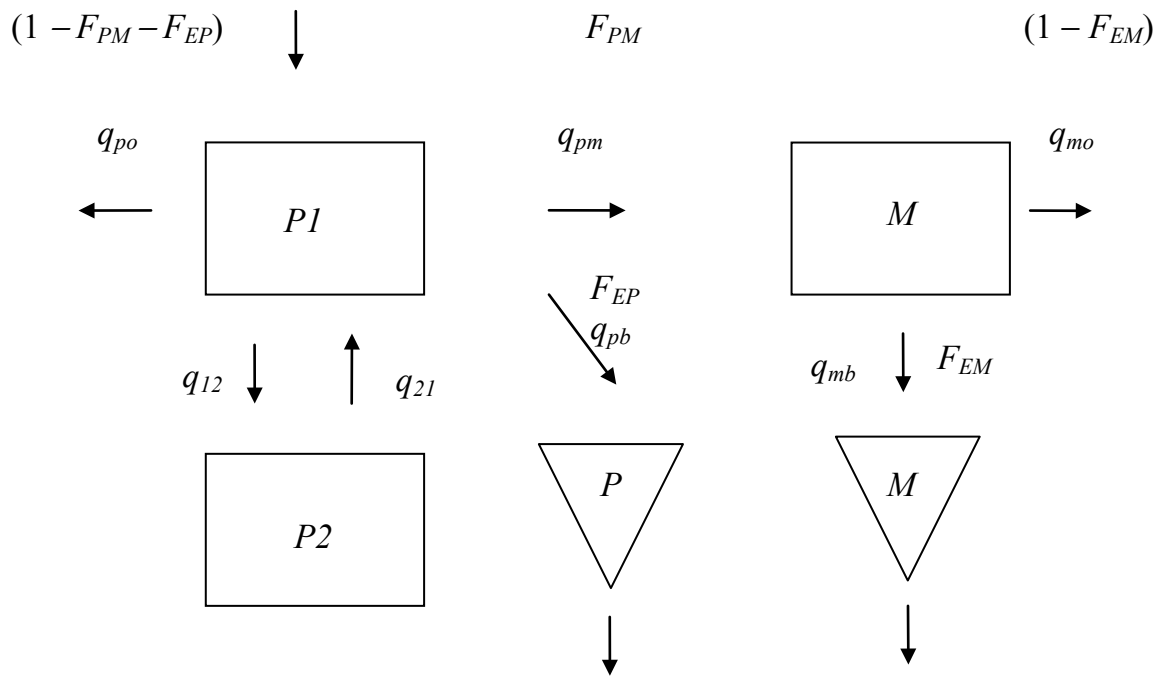


Figure 13.1 A 5-compartment pharmacokinetic parent-metabolite model for acepromazine ACP (*P*) and HEPS (*M*) in horses. Initially, the parent (*P*) enters into the first of two compartments, where it can move between this compartment (*P1*) and another (*P2*). A 2-compartment model was proposed for this part of the model as this was fitted in the analysis of ACP given by Ballard et al. (1982), Hashem and Keller (1993) and Marroum et al. (1994)

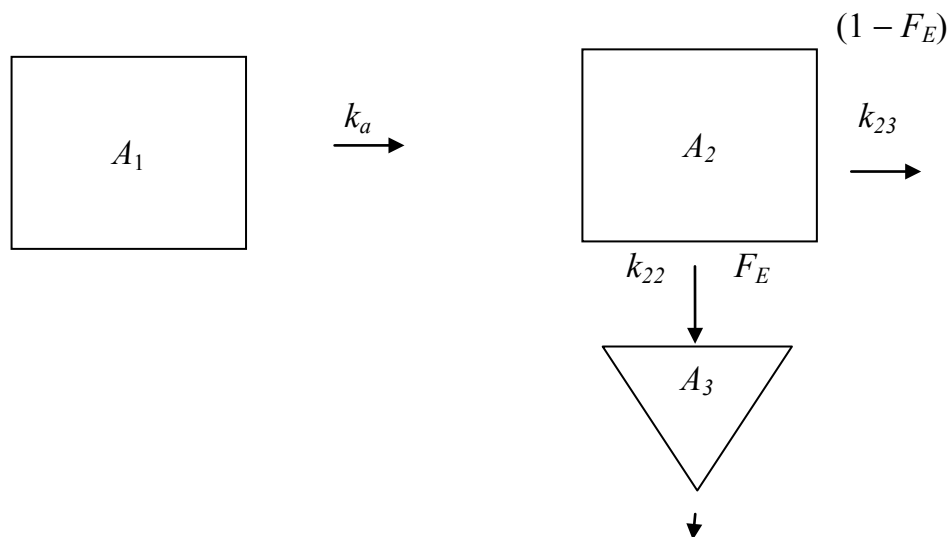


Figure 13.2 A simplified pharmacokinetic model for plasma concentrations and cumulative urine amounts of the metabolite 2-(1-hydroxyethyl)promazine (HEPS). Movement from compartment *A1* to *A2* represents the conversion of the parent (ACP) to the metabolite (HEPS) at rate k_a . Compartment *A2* represents the amount of HEPS in plasma with a fraction being excreted renally (F_E) to the bladder (*A3*) at a rate of k_{22} , and a fraction excreted non-renally ($1 - F_E$) at a rate of k_{23} . The metabolite will then accumulate in *A3* until the horse empties its bladder

Using this model we estimated the clearance rates from the renal and non-renal compartments, and the volume of distribution. As only the metabolite was modelled, the clearance and volume of distribution were scaled by the fraction of the parent converted to the metabolite. The ordinary differential equations used to describe the kinetics of this model were linear and hence were able to be solved analytically.

The probabilistic model

Having developed a structural model for the analysis, the next stage was to develop a full probabilistic model. Several alternative models were considered in order to describe the multivariate data in the simplest way possible. Instead of describing all the models considered here, we describe a ‘full’ model, such that all other rival models are parametrically nested within it. The development of this model can be described in four stages.

Stage 1

$$y_{ijk} | \lambda_{ij} \sim N(f_{ijk}, \gamma_{ijk})$$

where $f_{ijk} = f(\lambda_{ij}; t_{ijk}; D)$ represents the model prediction for the k^{th} response by the i^{th} individual on the j^{th} measurement, and $\gamma_{ijk} = \sigma_{\text{add}}^2 + \sigma_{\text{prop}}^2 f(\lambda_{ij}; t_{ijk}; D)^2$ for $i = 1, \dots, N, j = 1, 2$ and $k = 1, \dots, n_{ij}$.

Stage 2

$$\log \lambda_{ij} | \log \theta_i, \Phi \sim \text{MV } N_p(\log \theta_i, \Phi)$$

where θ_i represents the i^{th} individual’s mean PK behaviour and Φ is the measurement error variance-covariance matrix of PK parameters.

Stage 3

$$\log \theta_i | \log \mu, Z_i, \Omega \sim \text{MV } N_p(\log \mu, \Omega)$$

where Z_i represents the covariate values for the i^{th} individual, Ω the between horse variability and μ the population PK parameters.

Stage 4 (priors)

$$\sigma_{\text{add}}^2 \sim \text{Uni}(0, 100)$$

$$\sigma_{\text{prop}}^2 \sim \text{Uni}(0, 100)$$

$$\log \mu \sim \text{MV } N(0, 1000 \times I)$$

$$\log F_E \sim \text{Uni}(-300, 0)$$

$$\Phi \sim W(0.001 \times I, 5)$$

$$\Omega \sim W(0.001 \times I, 5)$$

where $\mu = [Cl, V, k_a]$ and $Uni()$, $MV N$ and W denote the Uniform, Multivariate, Normal and Wishart distributions, respectively. When estimating F_E , it is possible for this estimate to be larger than 1 through between subject variability and the variability due to measurement error. As this is not possible in practice, the constraint $\min\{1, F_E\}$ was imposed in the estimation. Further, k_a was assumed to be a fixed effects parameter, so no BSV was estimated.

Results and discussion

The concentration-time data and accumulated urine amounts of HEPS observed for the 12 horses are shown in Figure 13.3.

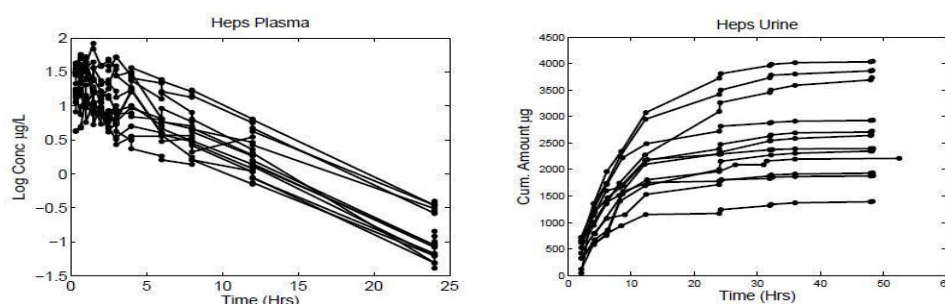


Figure 13.3 Observed plasma concentrations and urine amounts for the acepromazine metabolite HEPS after administration of 30 mg of acepromazine to 12 horses

Development and application of the final structural model

Several models were considered with a view to identifying the simplest model (that with the smallest number of parameters) that fitted the data best (as measured by the size of the deviance).

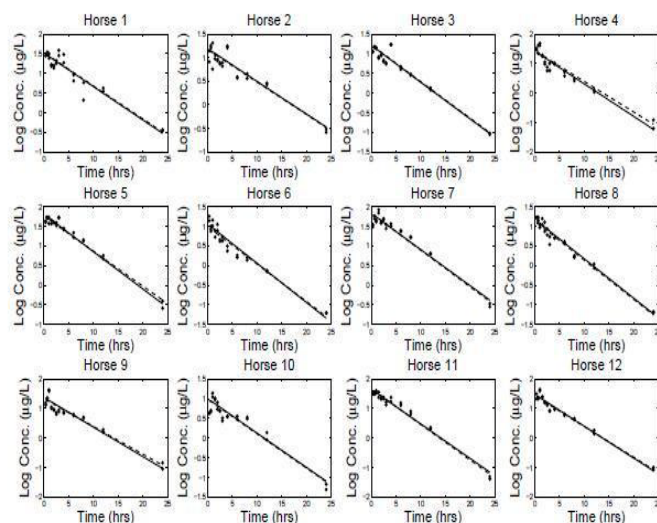
Additive and proportional residual errors were considered for each of the two responses (urine and plasma). The smallest mean deviance was obtained using a model that had a proportional residual error term on plasma concentrations of HEPS and an additive residual error term on cumulative urine amount of HEPS.

When the impact of measurement error was considered, it was found that including this variable in the model reduced the deviance from 2049 to 1977. This reduction was judged to be statistically significant based on a test devised by Spiegelhalter et al. (2002). Therefore, the variable measurement error (MEV) was retained in the final model.

The other covariates considered included age, bodyweight and breed. An initial examination based on the visual appraisal of several scatter-plots, suggested that a linear relationship may exist between bodyweight and volume of distribution. However, when bodyweight was included as an extra variable in the model there was no significant reduction in the deviance and further tests showed only a marginal effect overall. Thus, body weight was excluded from the final model.

Figure 13.4a and b shows how well the final model was able to fit the data for concentrations of HEPS in plasma and cumulative amounts of HEPS in urine over time, in the 12 individual horses.

(a)



(b)

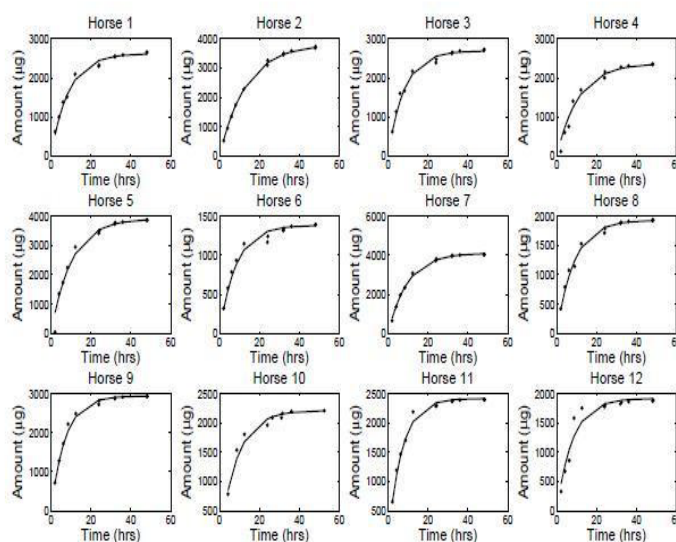


Figure 13.4 Relationship between time and concentration of the acepromazine metabolite HEPS in blood plasma (a) and urine (b) shown in 12 individual horses. The symbols represent individual (observed) data points and the lines represent a multivariate mathematical model constructed to describe these data. The dashed and solid lines shown on the plasma graphs (a) represent the independent application of the model to duplicate measurements made on the same blood samples

Figure 13.4a, where the dashed and solid lines overlap in most of the graphs, shows that the model provided a good representation of the data for both the duplicate measurements of HEPS in plasma, and confirms that overall the measurement error was low. Figure 13.4b shows that the model fitted the data well for the accumulation of HEPS in urine.

Individual parameter estimates derived from this model are shown in Table 13.2. At 769 L/h, the estimate of clearance rate, scaled by the fraction of ACP converted to HEPS, was found to be in reasonably close agreement with the estimate of 738 L/h (1.34 L/h/kg) that was derived using an independent modelling method as reported in Chapter 12. The large value estimated for k_a shows a rapid rate of conversion of ACP to HEPS. The between-subject variability was relatively small.

Table 13.2 Parameter estimates describing the pharmacokinetics of acepromazine (ACP) metabolite HEPS in 12 horses

		Between subject variability (BSV)	BSV %
Clearance rate (scaled) (Cl/F_{PM}), L/h	769	0.09	30
Fraction of HEPS excreted renally (F_E)	0.0856	0.11	32
Volume of distribution (scaled) (V/F_{PM}), L	6874	0.07	27
Conversion rate of ACP to HEPS (k_a), L/h	35.87	-	-
SD of proportional residual error, plasma concentrations (σ_p)	0.16	-	-
SD of proportional residual error, urine amounts (σ_u)	109	-	-

Clearance and volume of distribution were scaled according to the fraction of ACP that was converted to HEPS

Model validation

Residual error plots for the multivariate models are shown in Figure 13.5. For plasma concentrations, all plots appear to support the assumption of Normally distributed residuals. The QQ-plot shows some discrepancy between the standardized residuals and the fitted Normal density at the lower tail, but this was not deemed significant. Similarly, the residual plots for cumulative urine amounts generally do not violate the assumption for Normality. Again, discrepancies are apparent in the QQ-plot, but only at the tails.

Posterior predictive checks were used to determine whether the model developed was consistent with the data. The checks were performed in the following way. After fitting the final model, each parameter has a posterior distribution which summarized the uncertainty about the estimate (given the prior and the observed data). Ten thousand random samples were drawn from the posterior distribution, and the concentration ($\mu\text{g/L}$) at time t hours was simulated (based on each of the 10,000 random samples). This produced a distribution of concentrations ($\mu\text{g/L}$) at each time point. A 90% credible interval for these distributions can be seen in Figure 13.6.

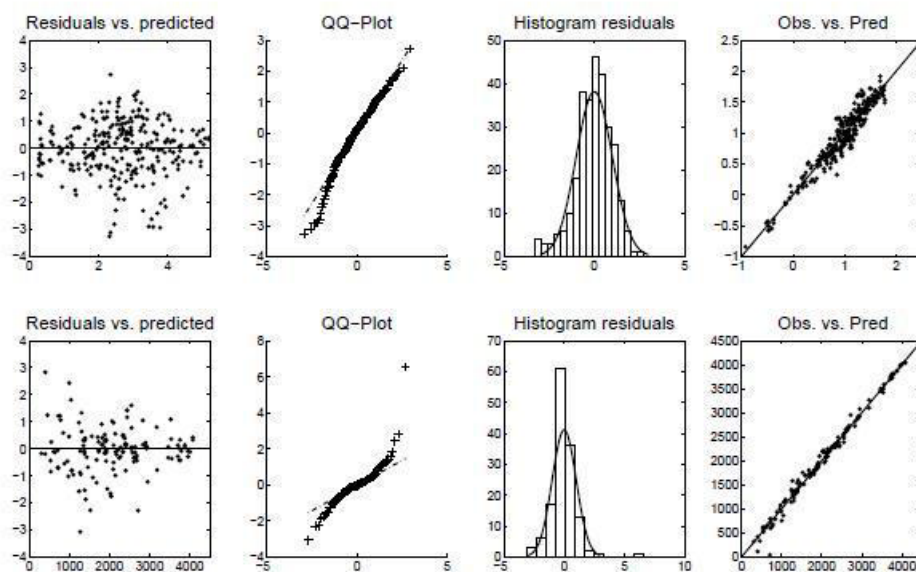


Figure 13.5 Residual error plots for a multivariate model describing the pharmacokinetics of the acepromazine metabolite HEPS in the blood plasma (top series) and urine (bottom series) of 12 horses

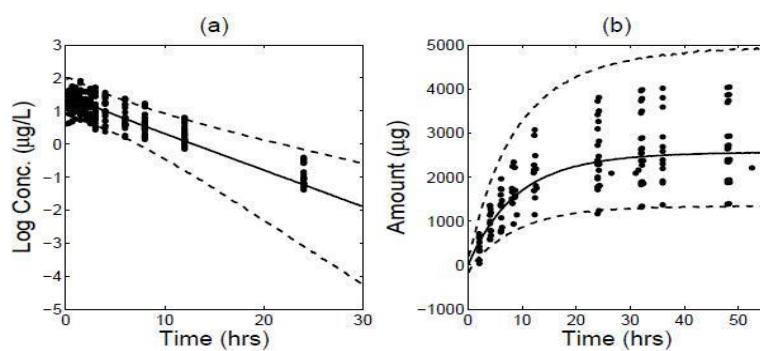


Figure 13.6 Posterior predictive check for plasma concentrations and urine amounts of the metabolite HEPS after the administration of acepromazine to 12 horses. The symbols represent actual observations, the solid line represents the multivariate model fitted to these data, and the dashed line represents a 90% credible interval for the model predictions

The plots in Figure 13.6 show that some of the observations fall outside the 90% credible interval. This is as expected, as approximately 10% of the observed data points should fall outside these bounds. For the plasma concentrations, the uncertainty below the median can be seen to increase with time, illustrated by the divergence of the solid and dashed lines. This is not surprising given that less data were available at later time points as the concentrations of HEPS in some samples fell below the limit of detection.

For the urine data, the upper bound of the 90% credible intervals extends noticeably higher than the observed data. This suggests that between-subject variation and/or measurement error might have inflated estimates of F_E leading to a larger than expected amount of HEPS being excreted renally. Thus, detection times may be longer than expected. Overall though, both posterior predictive checks seem consistent with the observed data.

Withholding and detection times

The detection time for a therapeutic substance represents the period after administration that the laboratories can identify the substance or its metabolite in either blood or urine. The withholding time is usually judged by a veterinarian and represents the period between drug administration and a race, taking into account the published detection time and a number of other factors. Toutain (2010) used Monte Carlo methods to estimate withholding times by extrapolating the detection times published by the European Horserace Scientific Liaison Committee. The methodology was based on a single exponential model for elimination and also relied on estimating a plasma-to-urine concentration ratio parameter. We feel that this methodology is inappropriate in the present case. The first reason is that the fraction of HEPS excreted renally (F_E) should be included in the estimation procedure. The population estimate for this parameter was 0.0856 indicating that only a relatively small amount of HEPS is excreted renally. Given the estimate is quite different from unity, it will likely have an influence on the estimates of detection times. A similar bioavailability term appears in the Toutain (2010) methodology. The second point is, given the model shown in Figure 13.2, it is likely that the plasma-to-urine concentration ratio is not constant for all time points. That is, the maximum plasma concentration is likely to occur before the maximum urine concentration, and therefore, assuming a constant ratio will provide biased results. The ratio based approach only seems reasonable for time points after the time of peak urine concentration. Lastly, an approach needs to be developed which allows for the horse to empty their bladder, as this is likely to occur in practice. Here we present an alternative approach to estimating withholding times based on the estimated uncertainty around the detection of the substance in plasma or urine at a given time point.

Probability of detection in plasma

Suppose we are interested in determining how likely it is for a horse to have a HEPS concentration in plasma or urine greater than the limit of detection (LOD) of 1 $\mu\text{g/L}$ at a certain time t after being given an intravenous bolus dose of 30 mg of ACP. Given the population models developed in this research, this can be resolved by considering the simulated data from the posterior predictive check.

In the posterior predictive check, random samples are drawn from the posterior distribution of parameters and data generated for some time points (t). Thus, the probability of a horse having a HEPS concentration greater than the LOD at time t can be determined from the simulated data. For a given time t , the number of simulated observations that are greater than the LOD is simply counted then divided by the total number of simulated observations at that particular time (t). This provides an estimate for the probability that a concentration will be larger than the LOD. A plot of these estimated probabilities can be seen in Figure 13.7.

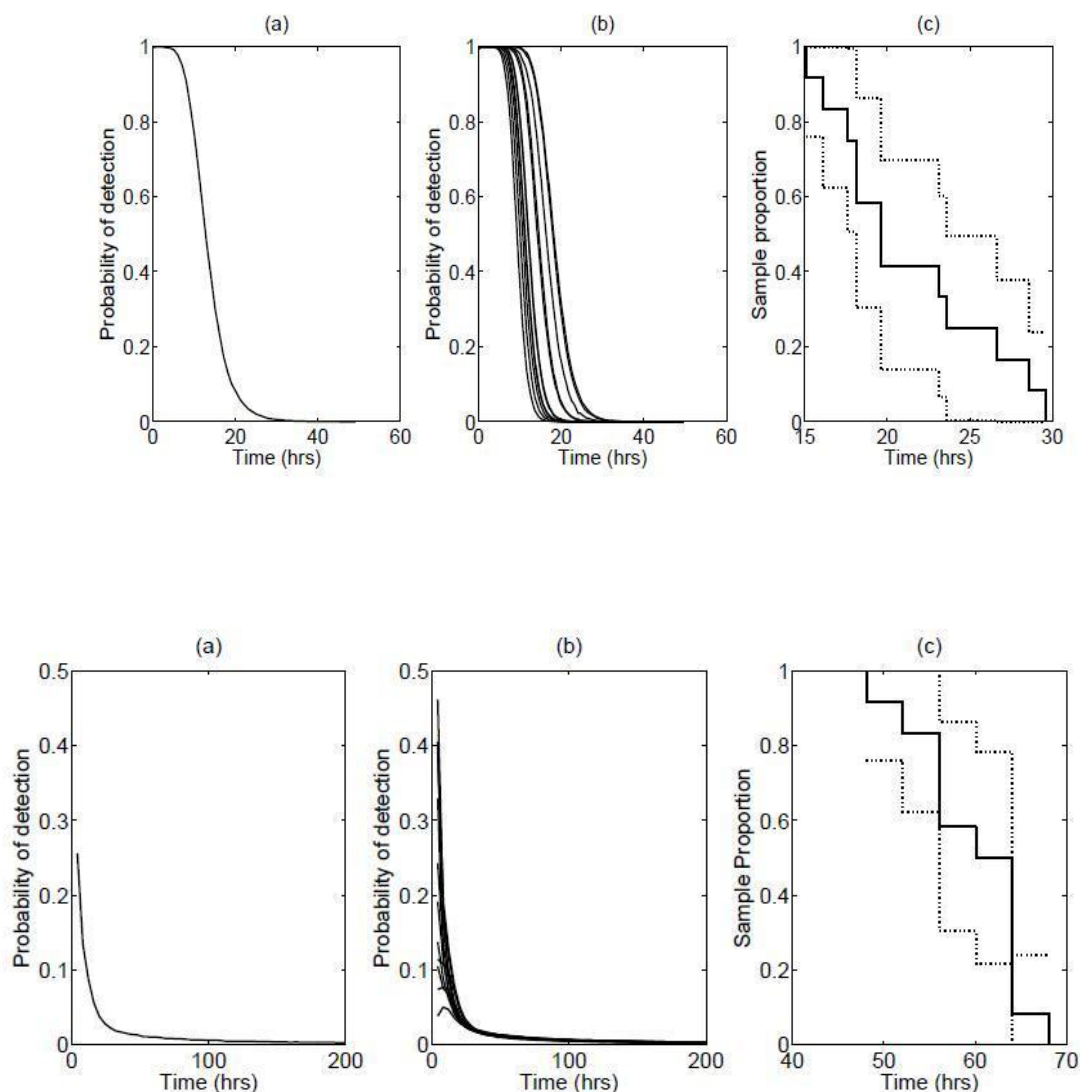


Figure 13.7 Probability plots for the detection of the acepromazine metabolite HEPS in plasma (top panel) and urine (bottom panel) after the administration of 30 mg acepromazine, and assuming a hypothetical limit of detection of 1 $\mu\text{g/L}$. Plot a illustrates the probability of detection over time for a typical horse in the population; plot b shows the estimated probabilities for the 12 individual horses used in the study; and plot c estimates the probability that a detection time is larger than a given time t (with 95% confidence bounds)

Figure 13.7, plot a, shows how the probability of breaching the detection threshold for a typical horse in the population decreases over time, as would be expected due to the constantly decreasing level of HEPS in the sample. Comparing the top and the bottom panel it can be seen that the detection period is much shorter in plasma than in urine, although in the latter samples there is still a low probability of detection past 50 hours, for example. This agrees well with the observed and predicted data shown in Figure 13.7b, as it appears that very little HEPS is accumulated in urine past this point in time.

Presenting information about detection times in this format could assist a person attempting to judge a suitable withholding time, as the figure offers a more detailed and realistic view of the data than the traditional approach of publishing a single detection time as x hours or days.

However, knowing the behaviour of a ‘typical horse in the population’ does not provide complete assistance in estimating the risk, as the degree of variability in the population should also be taken into account i.e. one needs to estimate the chance that any individual horse is in fact ‘typical’.

The degree of variation among the 12 horses used in this study is shown in Figure 13.7 plot b. Provided we assume that this particular group of horses was representative, this allows us to estimate the likely variation for this metabolite in the whole population (noting that the degree of variation may well differ from one drug to another).

Taking into account the variation seen with HEPS, plot c was constructed to illustrate the probability and (95% confidence interval around this probability), of a horse breaching the detection threshold at a given time. For example, the top panel (plasma) shows that at 30 hours after acepromazine administration, while only 10% of the horses would be expected on average to have a plasma concentration of HEPS greater than the LOD, in practice this percentage may be as small as 0% or as large as 25%. Similarly, for urine, Figure 13.7c (bottom panel) shows that on average, only 10% of horses might be expected to breach the detection level at 65 hrs, but in reality this estimate could lie anywhere between 0 and 25% for an individual horse.

Any estimate of a detection time will contain a certain level of uncertainty, and taking the degree of uncertainty into account for a particular drug or metabolite should assist in judging an appropriate withholding time.

A note concerning urinary estimates

To determine detection times in urine, an estimate of urine volume or accumulated urine volume at time t needed to be made. In the present study we observed a strong linear relationship between time and cumulative urine volume for all horses, and that the variability in this response increased with time.

This linear relationship allowed a linear mixed-effects model to be developed with the proportional residual error fitted as the independent variable. This model made it possible to predict the cumulative amount of HEPS and cumulative urine volume for a given time t . Given that the detection of any analyte in urine is based on concentration, these cumulative predictions needed to be combined and converted to concentrations.

In the present study we partitioned the data into 4 h windows from the time of drug administration. The amount of HEPS and urine volume accumulated in these windows was then used to predict concentrations. This approach required 6 assumptions to be made as follows.

1. Each sample was representative of the population;
2. the models developed describe the population;
3. a linear relationship exists between urinary excretion rate of HEPS and the production rate of urine;
4. the urinary production and excretion rate on race-day (and/or days before race-day) are the same as production and excretion rates during the study;
5. the bladder is completely emptied at the beginning of each 4 hour interval;

6. urine is collected at the end of a window.

Summary and conclusions

A Bayesian hierarchical model was used to describe the metabolism of acepromazine to HEPS. As the half-life of ACP was much shorter than that of the metabolite, the model was simplified to consider the metabolite only.

The structural form of this model for the description of HEPS concentration in plasma and cumulative amount in urine was based on mass balance, with vague priors chosen for the estimable parameters. No covariates were found to be statistically significant, but measurement variability in plasma concentrations proved to be influential, and random effects were included in the model to account for this.

Simulation techniques were used to show agreement between predicted and observed data, and also in the estimation of detection times for a typical horse in the sample. The results show how this novel approach could provide guidance for trainers and veterinarians to estimate appropriate withholding times with more confidence, ensuring that horses are not raced with prohibited substances in their system.

All inferences in this paper were based on a sample of 12 horses. While this is a larger sample size than that used in many previous pharmacokinetic studies, our conclusions still rest on the assumption that this particular sample is representative of the population at large, and so care should be taken when drawing any inferences from this work. Finally it should be noted that the detection times used to estimate probabilities are hypothetical and were used to illustrate the principles of this approach. They do not necessarily represent actual detection times currently in use in any racing jurisdiction.

Implications

This study has provided reliable pharmacokinetic data for 12 of the most commonly used equine medications across five therapeutic categories. This should assist clinicians in developing the most effective treatment regimes for sick or injured horses, regardless of whether the animals are competition horses or not.

For horses that may require treatment in the weeks or days leading up to a race, the data in this report will provide some assistance in judging appropriate withholding times. However, continued improvements in analytical sensitivity mean that an increasing number of drugs can be detected long after their pharmacological action has ceased. For therapeutic drugs, such detection capability must be constrained if drug control programmes are to avoid becoming a limiting factor in the proper veterinary care of competition horses.

In the short-term this report will be of assistance to the racing authorities in setting reliable detection times and appropriate reporting levels for therapeutic substances. Once these have been decided the information can be widely publicised. However, it should be noted that while this report provides accurate information on excretion times, no attempt has been made to recommend a detection time or reporting level for any particular substance.

Setting an appropriate reporting level where the period of detection far exceeds any likely therapeutic effect is a matter of optimisation. Regulators need to balance the risk of a horse competing under the influence of a therapeutic substance against the disadvantages of constraining the legitimate use of such substances, or preventing a treated animal from competing for an unreasonably long length of time. Providing a statistical decision-support framework to allow regulators to make such decisions in a rational and consistent way was beyond the scope of this project, but is entirely feasible if further work is supported.

Similarly, we have illustrated that for a given detection time, estimating an appropriate withholding time for the substance for an individual horse is a matter of risk management. A statistical decision-support framework to assist veterinarians in this regard would also be of value.

Finally, standardising detection capabilities between testing facilities is an important goal as equine sports become increasingly internationalised. To address this issue both the Fédération Equestre Internationale and the International Federation of Horseracing Authorities have adopted the principle of well defined analytical reporting criteria leading to realistic and internationally harmonised drug detection times. As these criteria will necessarily be based in large part on analyte concentrations in blood or urine, the development of accurate post-dose concentration data such as are presented here is vital to the process.

Recommended follow-up action

- The data in this report are intended to inform decisions made by the regulatory authorities regarding drug detection times and reporting levels. In all cases the reader is advised to refer to the relevant authorities for current guidelines and regulations concerning the use of therapeutic substances in competition horses.
- The results of six additional drug administration trials will be available in the next few months. This could be used to inform a follow-up report, or the completion of a body of work as a textbook or handbook;
- Data from these studies will be provided to the relevant regulatory authorities in Australia for review by racing analysts and veterinarians. It is recommended that current detection times are reviewed in the light of these data and amended where appropriate;
- The racing authorities could consider publication of any new guidelines concerning detection times in the form of industry fact sheets that are widely accessible;
- The authorities could consider sponsoring the development of a web site and/or application for mobile devices such as iPhone and iPad, to make the relevant data readily accessible, as has been achieved in Europe;
- The peak industry bodies (as represented on the ETRA reference group) could assist in:
 - raising awareness of the availability of this report via RIRDC,
 - disseminating relevant information contained in this report, and
 - disseminating any new information published by the racing authorities with respect to new detection times;
- Now that accurate information on excretion rates is available for several long-acting substances, the authorities could consider sponsoring the development of a statistical decision-support framework to allow detection times or reporting levels to be optimised on a rational basis. An optimised reporting level would balance the risk of a horse competing under the influence of a therapeutic substance, against constraining the legitimate use of such a substance for an unreasonable length of time.

Glossary

AA	aminoantipyrine
ACTH	adrenocorticotrophic hormone
AUC	area under plasma-concentration versus time plot
AUMC	area under the first moment versus time curve
BHM	Bayesian hierarchical model
bid	twice daily
BSV	between-subject variability
BW	bodyweight
°C	degrees Celsius
Cl	total body clearance rate
C _{max}	peak plasma concentration
COX	cyclo-oxygenase
CSU	Charles Sturt University
FAA	formylaminoantipyrine
G	gauge
g	acceleration of gravity
g	gram
GX	glycinexylidide
h	hour
HEPS	2-(1-hydroxyethyl) promazine sulphoxide
HBB	hyoscine-N-butyl bromide
IM	intramuscular
IV	intravenous
kg	kilogram
L	litre
LOD	limit of detection
LOQ	limit of quantification
MAA	4-methylaminoantipyrine
MCMC	Markov chain Monte Carlo
MEV	variable measurement error
MEGX	monoethylglycinexylidide
μL	microlitre
μg	microgram
mg	milligram
min	minute
mL	millilitre
mM	millimolar
MRT	mean residence time
MV	multivariate distribution
ng	nanogram
N	Normal distribution

NSAID	non-steroidal anti-inflammatory drug
OBJ	NONMEM objective function value
OPB	oxyphenbutazone
PBZ	phenylbutazone
PD	pharmacodynamics
PK	pharmacokinetics
PPID	pituitary <i>pars-intermedia</i> dysfunction
RUV	residual unexplained variability
SC	subcutaneous
SD	standard deviation
se	standard error
sid	once daily
T	time
$T_{1/2}$	terminal half life
U/P	urine-to-plasma ratio
UQ	The University of Queensland
Vd	volume of distribution
VPC	visual predictive check
Vssc	volume of distribution at steady state
W	Wishart distribution

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The Pharmacokinetics of Equine Medications

by Martin Sillence, Glenys Noble, Fiona Schneiders, Wayne Bryden, Judy Cawdell-Smith, Melody de Laat, Mark Jarrett, Bruce Young, Andrew McKinney, Adam Cawley, Jessica Booth, John Vine, Linda Glowacki, James McGree, Ray Boston, Samantha Nelis, Carl Kirkpatrick, Nick Shaw, and Barry Smyth

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This report provides information about the pharmacokinetics of 12 equine medications. For each medication, the report illustrates that time taken for the drug to reach its maximum concentration (and hence maximum effectiveness) in the blood, its rate of metabolism and removal from the bloodstream, and the time taken for the drug and its metabolites to be eliminated from the body via the urine.

The report also provides new insights into how metabolite data can be modelled to provide useful information about the behaviour of the parent drug, and how the variation between individual horses can be measured and used to estimate the level of risk of any horse in the population of breaching a detection limit at any given time point after drug administration.

This information should assist veterinarians to develop more effective treatment protocols for horses, and will assist the racing industry regulators in setting appropriate detection times and reporting levels for therapeutic substances in animals that compete.

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